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14. ABSTRACT

The Integrative Cardiac Health Project (ICHP) aims to lead the way in Cardiovascular Disease (CVD) Prevention by conducting novel research utilizing a Systems Biology / personalized medicine design to discover and develop practical, effective and preemptive integrative approaches in order to detect and combat CVD earlier before it affects the quality of life. ICHP's ultimate goal is to translate our evidenced-based research findings for application into clinical practice. A translational research approach will provide the ability to find novel disease markers, optimal prevention and holistic treatment approaches, and a unique venue for future research as the "virtual laboratory" for optimal comprehensive health prevention in the military beneficiary population. This research method also allow us to further hypothesize and define relationships between CVD, other cardio metabolic disease states and maladaptive behavior patterns unique to service members such as pre-diabetes, stress, overweight and sleep disorders with the aim of targeting these disorders in a pre-clinical phase. Using an integrative, interdisciplinary preventive health approach, ICHP has shown that an individual's cluster of CV risk factors can be effectively targeted and improved.

15. SUBJECT TERMS
Lifestyle; Cardiovascular Disease (CVD); Prevention; Behavior; Coronary Heart Disease (CHD); Genomics; Metabolic Syndrome; PreDiabetes; Diabetes; Obesity; Stress

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Integrative Cardiac Health Project (ICHP) Annual Report
Executive Summary
Dates: 30SEP13 – 28SEP14

ICHP aims to lead the way in Cardiovascular Disease (CVD) Prevention by conducting novel research to discover and develop practical, effective and preemptive integrative approaches to detect and combat CVD earlier before it affects the quality of life. ICHPôs ultimate goal is to translate our evidenced-based research findings for application into clinical practice. In the past year, these key accomplishments are noted:

- Total yearly visits at WRNMMC ICHP: 1976 (includes follow-up motivational coaching calls)
- Protocols: Active ï 15 (5 closed to enrollment); Closed ï 5 (2 in publication submission); Submitted-1; In preparation-1.
- *Dissemination of scientific research findings continues*:
  - 3 manuscript published; 7 in preparation
  - 1 abstracts published; 2 abstracts presented as posters
  - 1 abstract accepted for poster presentation; 8 abstracts submitted
- Scientific Advisory Board meeting held December 2013
- ICHP manuscript included as evidence in new AHA/ACC Clinical Guidelines for CVD Risk Assessment
- ICHP Clinical Decision Support Tool developed and translated into practice to improve CVD risk classification
- Development and implementation of an Executive Health Program to address issues relevant to military senior leadership
- ICHP Database and Platform Creation nearing completion.
- Data analysis continues. Relevant findings in our population during the past year include:
  Sleep: In a moderate-sized cohort of subjects with obstructive sleep apnea verified by polysomnography, women experienced fatigue more commonly than did men even when objective measures of OSA severity were similar.
  Pre-Hypertension: CVD risk factors appear to cluster in subjects with prehypertension, placing these subjects at increased risk for CVD much like subjects with hypertension.
  Lipoprotein Insulin Resistance Index (LP-IR): LP-IR is described as a reliable biomarker for progression to diabetes that reflects improvements in metabolic syndrome following dietary/lifestyle interventions with weight loss. The majority of ICHP individuals who lose weight reduce their LP-IR. However, a subgroup (25%) of patients increased their LP-IR despite weight loss. The clinical and prognostic significance of these observations require further study. Additionally, Lifestyle modification including a Mediterranean diet is comparable to a stringent intervention with a vegetarian diet for improving insulin resistance defined by LP-IR. Significant reductions in large VLDL/chylomicrons may drive improvement in IR irrespective of dietary stringency.
  Prediabetes: A comprehensive health program emphasizing combined improvements in nutrition, exercise, stress and sleep may help subjects with prediabetes revert to normal glucose metabolism without substantial changes in BMI. Combatting progression to diabetes with a practical lifestyle intervention lowers CVD risk and improves overall health in this vulnerable population.
  Weight loss and gene expression: Widespread gene expression changes associated with vascular immune and inflammatory responses were associated with substantial but not minimal weight loss during intensive lifestyle modification for cardiovascular risk reduction.
  Lifestyle program attrition: Clinical trials and lifestyle programs for cardiovascular risk reduction should recognize that personal barriers to continued participation differ between women and men and must strive to accommodate all barriers in order to maximize patient retention.
- Upon receipt of the no-cost extension approval ICHP terminated the 500K vendor contract with Theranos. The funds were moved to support the ICHP Personnel category. The program elected to close an additional vendor contract with Theranos as well as a subaward with Geneva Foundation. These efforts have been taken to sustain the program through the no-cost extension period.

Introduction

In 1998, Congress supported the need for basic and clinical research in Coronary Artery and Prostate Disease in order to reduce the incidence of these life-threatening diseases and develop more effective, more specific, and less invasive forms of therapy for patients (Public Law No. 105-262). In FY10, the Integrative Cardiac Health Project (ICHP) was identified as a cardiovascular (CV) research Center of Excellence (COE) by Health Affairs and placed into the Army Program Objective Memorandum (POM). ICHP continues its operation at the Walter Reed National Military Medical Center (WRNMMC) in Bethesda, Maryland.

Cardiovascular Disease (CVD) risk is at epidemic proportions in the Military Health System (MHS). Heart disease is the most common, costly, and preventable of all health problems and the MHS has a large number of beneficiaries at risk for CVD. Cardiac related events make up a significant portion of non-battle disease injuries requiring evacuation from Theater jeopardizing operational effectiveness. Service members with multiple combat deployments and Wounded Warriors have increased CVD morbidity/mortality risk (2 and 3.5 fold, respectively).[1,2] Despite optimal medical therapy such as statins, there remains a residual CVD risk of approximately 69%.[3] Existing wellness programs in the Department of Defense (DOD) and civilian healthcare do not adequately address CVD risk or obstacles related to healthy living which contribute to escalating CVD risk. This large gap in care can only be addressed with innovative, intensive, multi mechanistic approaches to improve CVD outcomes. There is a critical need for personalized CVD risk reduction and actionable empowerment strategies/tools to optimize health and reduce cost.

The ICHP (HJF) champions the way for optimal CV Health in the MHS by conducting novel research utilizing a Systems Biology/personalized medicine design to discover and develop practical, pre-emptive and integrative approaches in order to detect and combat CVD earlier and augment traditional care before it affects the quality of life. This vision is in support of the Military Healthcare System (MHS) Strategic Focus and Quadruple Aim on health and wellness and complements the Army Performance Triad. ICHPôs research impacted the 2013 American Heart Association (AHA)/American College of Cardiology (ACC) Prevention Guidelines[4] and one of ICHPôs prevention pilot models has been translated into practice complementing the US Army Surgeon Generalôs Executive Health and Wellness Program.

ICHPôs ultimate goal is to translate our evidenced-based research findings for application into clinical practice in an effort to achieve the following research aims:

- Improve Force Health by better understanding the CVD risk susceptibility of military specific populations as well as to understand the individual service member through leading-edge research using novel tools and technologies.
- Investigate and create transformational models of healthcare delivery through personalized CVD prevention tracks as an adjunct to traditional care.
- Refine individualized prevention strategies through statistical data modeling to define the most cost-effective and sustainable approaches in promoting cardiovascular health throughout the military lifecycle.
- Simultaneously, improve understanding of the molecular, physiological, biochemical, immunological and environmental basis of cardiovascular (CV) health and disease and to

use that understanding to develop improved approaches to disease diagnosis, treatment and prevention, in line with NHLBI Strategic Plan 2008.

Body

Overall Program Initiatives

On 6 Dec 2013, ICHP held its second meeting of their Scientific Advisory Board (SAB) in which current research initiatives were reviewed and new directions of research and translational opportunities were examined. This successful meeting validated ICHPôs future scientific roadmap on both the clinical and molecular level.

The following significant ICHP achievements should be highlighted in this report. An ICHP manuscript[5] was included as evidence to support the new Clinical Guideline change to include family history as a significant CVD risk factor by the American Heart Association and American College of Cardiology Expert Panel 2013 for New Guidelines in CVD Risk assessment. Additionally, upon request of the OTSG of the Army, ICHP has developed a customized model for Executive Health to address issues relevant to our nationôs leaders (stress, travel and jet lag). Two interactive and educational workshops along with personalized lifestyle prescriptions for each leader and/or spouse were provided with a high level of satisfaction from the Surgeon General. Lieutenant General Patricia Horoho recognized ICHPôs full support of the MHS strategic focus on Health and Wellness, when she stated that ñICHP provides a phenomenal model for initiating integrative wellness programs throughout the military. The evidence-based approach of the ICHP team compliments military medicine.ò In her testimony to Congressô House Appropriations Committee on April 2, 2014, LTG Horoho stated ñICHP is the only COE that specifically addresses obstacles related to healthy living in the military. ICHP is synchronized with Army Medicineôs movement to improve health.ò[6]

To better reflect ICHPôs role in overall warrior health, ñCardiovascular Prevention Program (CPP)ò has been changed to ñCardiovascular Health Program (CHP)ò. Marketing materials, program forms and protocols revisions to reflect the new name are in progress.

The following staff additions in the past year will be instrumental in our ability to move forward with our current research portfolio as well as in the design of new and novel science:

- ❑ Cardio-Immunology Physician Consultant with an expertise in inflammatory markers as predictors of atherosclerotic disease will be instrumental in designing future ICHP protocols.
- ❑ Outcomes Data Specialist will pursue the ICHP Data Management Plan to merge data from two previous databases in order to move forward with one dataset for further analysis, including 100% quality assurance of data.
- ❑ Sonographer will conduct both carotid ultrasound and echocardiograms for protocols.

In the past quarter, the ICHP Executive Team was actively engaged in the submission of our FY 2015-2019 research proposal. This submission includes ongoing ICHP research but also the design of a new ICHP randomized, controlled landmark protocol by the cardio-immunologist physician consultant with a focus on gender and biomarkers as predictors of atherosclerotic disease.

Task #1: Complete the "*B*etter Adherence *to T*herapeutic *L*ifestyle Change *E*fforts (BATTLE) Trial".

Methodology

The purpose of this study is to determine whether knowledge of abnormal results from a noninvasive test for detection of subclinical atherosclerosis (CIMT), in addition to knowledge of CVD risk factors, enhances adherence to healthy lifestyle behaviors in comparison to only CVD risk factor knowledge. The study will be conducted with individuals at moderate to high risk for cardiovascular events based on CVD risk factor profile and evidence of significant subclinical atherosclerosis.

This two-arm, double-blinded study will randomize subjects to either receive CIMT results (R-CIMT Group) or have CIMT results withheld (W-CIMT Group) in the setting of a 3-month TLC intervention. After the 3-month TLC intervention period is completed, subjects who had CIMT results withheld will receive this information. Because knowledge of the study hypothesis could impact their behavior during the lifestyle intervention, subjects will be blinded to the study hypothesis. Similarly, research staff implementing the TLC intervention will be blinded to subjectsô randomization assignment.

It is hypothesized that participants with CVD risk factors who have knowledge of their own CIMT test results showing significant subclinical atherosclerosis will demonstrate better adherence to TLC than those subjects from whom the CIMT test information is withheld. A composite index of adherence to the TLC intervention was selected as the primary outcome measure since the main goal of this study is to assess the impact of CIMT imaging knowledge on change in lifestyle behaviors.

A combined measure of adherence, reflecting both aspects of the lifestyle intervention (Mediterranean-type diet, moderate aerobic exercise), was chosen that uses accepted measures of diet and exercise adherence reported in the literature. Secondary outcomes include: 1) Adherence to each program components; 2) Changes in modifiable CVD risk factors and other biochemical markers; 3) Emotional factors such as anxiety, self-efficacy, motivation, and 4) Atherosclerosis and CIMT Knowledge Assessment Score (only in CIMT-R subjects).

Results/Conclusions:

Key study findings were reported in the W81XWH-11-2-0227 (FY12-14 Yr 1) Annual Report dated October 27, 2012.

Status: Manuscripts are being finalized for submission. Study closure documents were approved by WRNMMC IRB on 25 October 2012 and forwarded to USAMRMC HRPO.

Manuscripts in preparation:

- ❑ Saum NS, Halsey JF, Walizer EM, Vernalis MN. Exploring the role and impact of limited mindfulness training in changing diet and exercise behaviors. (In preparation)

- ❑ Walizer EM, Vernalis MN. Does visual knowledge of increased risk for cardiovascular disease affect lifestyle change program adherence? (In preparation)

Abstract Published/Presented as Poster:

Walizer EM, Vernalis MN, Modlin RE. Influence of CIMT as a motivator for health behavior change in a heart health program. *Circ.* 2014;129:AP126. (*AHA EPI/NPAM 2014 Scientific Session*, San Francisco, CA, March 19, 2014)

Abstract

Introduction: Carotid intima media thickness (CIMT) ultrasound is a known surrogate marker of atherosclerosis but few studies examine its influence on patient behavior. Motivation and self-efficacy (SE) are known predictors of health behavior change. This randomized, double-blind trial examined 1) use of CIMT images plus associated CVD risk to motivate adherence, and 2) the predictive ability of motivation and SE on adherence change.

Methods: Patients with Ó2 cardiovascular disease (CVD) risk factors and subclinical atherosclerosis were assigned to either the intervention group [receive results weekly (R-CIMT)] or control group [withhold results (W-CIMT)]. All patients received a 12-week lifestyle program (Mediterranean diet, aerobic exercise, group support). Overall change in adherence from baseline to week 12 was determined using an ANCOVA model where % adherence was a composite measure of diet and exercise adherence. Initial motivation plus exercise and nutrition SE were assessed to determine their predictive ability of adherence in a standard regression model.

Results: 166 patients randomized; 161 (R-CIMT n=81; W-CIMT n=80) eligible for intention-to-treat analysis. Patients were middle age (mean age = 54 Ñ11 yrs), 62% (100 or 161) women, 48% (77 of 161) black. Baseline group differences: W-CIMT group was younger (52 vs 55 yrs; p=0.05), had a lower systolic blood pressure (120 vs 125; p=0.01), lower % family history of CVD (49 vs 65; p=0.03). In comparing R-CIMT vs W-CIMT groups, no difference was detected in overall % adherence change (16.4 Ñ25.6 vs 19.8 Ñ25.4; p=0.39). Initial motivation and SE measures were not predictive of change in adherence when added to group assignment (see Table).

	P-Value (Regression Coefficient)			
Model	CIMT Group	Exercise SE	Nutrition SE	Initial Motivation
Adherence Change* = CIMT group + Initial Motivation	0.443			0.677
Adherence Change* = CIMT group + Exercise SE[1] + Nutrition SE[1]	0.414	0.927	0.784	
Adherence Change* = CIMT group + Initial Motivation + Exercise SE[1] + Nutrition SE[1]	0.469	0.798	0.575	0.552
*Adherence change was computed by adherence (capped at 100%) at week 12. [1]Baseline exercise and nutrition SE are considered covariates.				

Conclusions: CIMT evidence of subclinical atherosclerosis increased participant CVD risk awareness but did not translate into actionable healthy behavior changes beyond those in the control group. Neither exercise nor dietary adherence was affected by initial motivation or self-efficacy when added to CIMT risk awareness.

Task #2: Complete the CADRe Five-Year Follow-up Protocol.

Methodology

This follow-up study will determine the persistence of healthy lifestyle behavioral changes and CVD risk factor control results after their original CADRe study participation. This study will continue as a longitudinal observational study where patients will have yearly follow-up visits at 1, 2, 3, 4, and 5 years after completion or expected completion of the CADRe Study. This study will involve prospective collection of data. All collected data is considered WRNMMC Cardiology standard of care for the study population identified.

It is hypothesized that participants who have been exposed to an intensive lifestyle change program will demonstrate long-term carryover of heart healthy characteristics including persistence of favorable lifestyle change behaviors and risk factor control. Up to 163 male and female CADRe study participants, age 18 years or older, with subsequent completion of Phase 1 of the CADRe Study (3-month data collection) were re-contacted and invited to participate in this 5-year follow-up study (post-study completion or expected completion).

A composite index of 7 heart healthy characteristics (BMI 18.5 ï 25; LDL-cholesterol < 100 mg/dL; dietary fiber intake Ó25 gms/day; consumption of 5 or more fruits and vegetables per day; BP < 140/90 mmHg; regular exercise Ó150 min/week, and daily practice of CADRe program stress management techniques) was selected as the primary outcome measure since the main goal of this study is to assess the persistence of lifestyle change behaviors and risk factor control. The Heart Health Index (HHI), presented as a single score (range 0-7), will be assigned to each subject yearly. Additionally, each of the 7 heart healthy characteristics will be assessed independently as a continuous variable. Secondary outcome measures include: Changes in modifiable CVD risk factors (blood pressure, body composition and fitness, lipid levels and glucose); C-reactive protein and, Quality of Life.

Results/Conclusions:

Key study findings were reported in the W81XWH-11-2-0227 (FY12-14 Yr 2) Annual Report dated October 29, 2013.

Status: Study closure documents approved by WRNMMC DRP on 12 March 2013 and approved by USAMRMC ORP HRPO on 17 May 2013. Manuscript preparation is in progress.

Task #3: Continuation of the "Comprehensive Cardiovascular Risk Assessment and Prevention Program (CHP)" at WRNMMC.

Methodology

This program serves as a platform for ongoing translational research activities, a ñvirtual laboratoryò based on scientific findings for the development of best personalized preventive practices. In other words, the platform allows ICHP to gather an expansive number of data points for each patient or subgroup of patients (eventually combined with data at a molecular level) that when leveraged will result in the creation of new tools in technology to define wellness, predict and prevent disease, and empower patients and providers to transform their healthcare.

The CHP platform has a dual purpose and is multifunctional. This platform 1) allows for multiple research protocols to be conducted as it sets the stage for recruitment, enrollment and

hypothesis generation, advanced data modeling and simultaneously 2) provides a venue where research findings from these protocols can then be tested, validated and translated into application for clinical practice. Our protocols within the CHP are specifically designed to examine the effects of our militaryôs high op tempo which predisposes our service members to accelerated atherosclerotic risk resulting from high stress, PTSD, depression, sleep insufficiency, overweight, prediabetes and prehypertension among other traditional disease risk factors.

This program was established to address the unique needs of military beneficiaries at risk for CV disease. It includes conventional and novel CV risk profiling (health assessments, labs, markers, wearable monitors) along with tailored and personalized behavioral recommendations for primary or secondary prevention by an integrative team of providers comprised of a cardiologist, sleep specialist, nurse practitioners, nutritionists, stress management instructors and exercise physiologists. Validated tools to screen for and measure CV risk are part of this inclusive package. Report cards for the patient and provider as well as email notifications are utilized. The program is an adjunct to the best medical practices provided by their primary care provider. Up to 1000 patients may be enrolled each year. Some of the patients (such as nurses or traumatic injury patients, etc.) may be in subgroup programs because of unique needs. The CHP serves as a platform for ongoing translational research activities, a ñvirtual laboratoryò for the development of best preventive practices and for CV educational and marketing materials.

The ñOutcomes of the CPP Programò protocol provides for retrospective examination of existing data for the purpose of examination and reporting of the results of the evaluations and interventions of the CHP. The annual continuing review (CR) was approved by WRNMMC IRB on 22 Apr 14. A Change of PI amendment from COL Randolph Modlin, MC, USA to LTC Todd Villines, MC, USA was submitted to WRNMMC IRB and approved as of 6 Aug 2014. These approvals were forwarded to HRPO via HJF.

Status:
Total patient visits during past year: 1976 (includes telephonic coaching calls)
Manuscripts-Published (See Appendix A):
- Eliasson A, Kashani M, Modlin R, Howard R, Vernalis M. Fatigued on Venus, Sleepy on Marsð Gender and racial differences in symptoms of sleep apnea. *Sleep Breath.* 2014 Mar 15. [Epub ahead of print]

Manuscript-In-preparation:
- Kashani M, Eliasson A, Modlin R, Vernalis M. Cardiovascular Health Program Increases Self-Efficacy.

Abstract Accepted for Poster Presentation:
- Eliasson AH, Kashani MD, Doody MM, Jones MK, Vernalis MN. Fatigue in Women is a Key Symptom in Evaluation of Sleep Apnea. *CHEST*, Oct 2014; Austin, TX.

Abstract

Purpose: Recently published guidelines for management of obstructive sleep apnea (OSA) endorse evaluation of sleepiness with the Epworth Scale but do not suggest the assessment of fatigue. Prior research on gender differences in OSA symptoms has shown conflicting results in part because symptom questionnaires have not included fatigue and in part because OSA was

determined by screening questionnaire not by gold standard overnight polysomnography. We sought to clarify if symptoms differed by gender in subjects with OSA confirmed by overnight polysomnography utilizing symptom-specific questionnaires.
Methods: Of subjects entering a cardiovascular disease prevention registry, we gathered data on demographics and sleep-related symptoms for consecutive patients who underwent diagnostic polysomnography. OSA was defined with a respiratory disturbance index (RDI) of Ó5 events per hour. Sleepiness was recorded using the Epworth Scale (ES, range 0 to 24). Fatigue was measured with the Stanford Fatigue Scale (FS, range 0 to 10). Subjects with and without OSA were compared by gender for symptoms of fatigue and daytime sleepiness by t-test.
Results: Of 62 consecutive subjects (40 women, mean age 57.4Ñ12.6 years) ES was 10.0Ñ4.7, FS was 4.9Ñ1.9. With no OSA, ES in women (8.2Ñ4.8) was not different from men (9.5Ñ4.0, p=0.63) and FS in women (5.4Ñ2.0) was not different from men (5.0Ñ3.2, p=0.79). However, with OSA, ES in women (10.4Ñ5.3) was similar to men (10.5Ñ5.0, p=0.93) but FS in women (5.4Ñ1.8) was significantly higher than for men (4.1Ñ1.8, p=0.03). This greater degree of fatigue in women with OSA was found despite a lack of statistical difference in polysomnographical variables between women and men for RDI (p=0.42), arousal index (p=0.10), and %time<90% saturated (p=0.18).
Conclusions: In this moderate-sized cohort of subjects with OSA verified by polysomnography, women experienced fatigue more commonly than did men even when objective measures of OSA severity were similar. This finding broadens our understanding or how genders manifest symptoms of OSA differently.
Clinical implications: Providers can better capture OSA in women by using the proper questionnaire tool to screen for fatigue, not relying solely on assessments of sleepiness. Future clinical guidelines should incorporate this recommendation to avoid under-recognition of sleep pathology in women.

Abstract Submitted for Poster Presentations:

- Kashani M, Eliasson A, Engler R, Fuller C, Vernalis M. Prehypertension coexists with CVD risk factors. *AHA Scientific Session 2014*. November 2014.

Abstract

Introduction: The most recent guidelines for the management of blood pressure (BP) solely address hypertension, no longer considering the classification of pre-hypertension. Subjects who do not qualify as hypertensive but have a modest increase in BP are unrecognized despite their potential for cardiovascular disease (CVD) risk.
Objective: We examined the CVD risk profile of subjects with modest BP elevation to determine the prevalence of risk factors and to identify targets for intervention.
Methods: Consecutive subjects entering the Integrative Cardiac Health Projectôs 12-month CVD Risk Reduction Registry were assessed for anthropometrics and a CVD-relevant lab panel. Subjects were categorized as normotensive (BP≤120/80), pre-hypertensive (BP>120/80 and <140/90) and hypertensive (BP≥140/90). We compared normotensive subjects with pre-hypertensive and hypertensive subjects for differences in CVD risk factors using t-test.
Results: Of 352 subjects (56% women, mean age 53 Ñ13.5 years, 61% white, 22% black, 5% Hispanic), 114 (32%) were normotensive, 154 (44%) pre-hypertensive and 84 (24%) hypertensive. For the parameters above, there were no differences between the hypertensive group and the prehypertensive group.

	BP mmHg	Gluc mg/dL	HOMA	HbA1C %	LDL mg/dL	HDL mg/dL	TG mg/dL	BMI kg/m^2	WC cm
Normotensive	112/72	93.9 Ñ16.7	2.89 Ñ2.6	5.5 Ñ0.6	108.5 Ñ28.7	60.4 Ñ17.0	97.6 Ñ50.7	28.2 Ñ5.8	94.3 Ñ15.1
Prehypertensive	128/80	100.6 Ñ14.9	3.75 Ñ3.8	5.7 Ñ0.6	115.0 Ñ 38.0	55.2 Ñ13.6	115.7 Ñ66.1	30.5 Ñ5.5	102.8 Ñ14.1
p value		0.001	0.01	0.02	0.12	0.009	0.012	0.0006	0.0001

Gluc = glucose, HOMA = homeostatic model assessment, HbA1C = hemoglobin A1C, BMI = body mass index, WC = waist circumference.

Conclusion: CVD risk factors appear to cluster in subjects with prehypertension, placing these subjects at increased risk for CVD much like subjects with hypertension. In particular, dyslipidemia and glucose dysmetabolism co-exist with very modest increases in BP, laying a foundation for metabolic syndrome. In applying current BP guidelines to this population of predominantly middle-aged women, subjects would be left vulnerable and unrecognized for their increased risk. Such subjects deserve close scrutiny for their comorbid risk factors and interventions with targeted lifestyle changes.

Abstract Submitted for Poster Presentations:

- Engler, RJ, Vernalis MN, Mamula KA, Blackburn HL, Kashani M, Ellsworth DL. Lipoprotein Insulin Resistance Index (LP-IR) Changes with Weight Loss Following 1 Year Low Fat Vegan Diet. *American College of Cardiology, 64th Annual Scientific Session*, March 14-16, 2015, San Diego, CA.

Abstract

Background: Lipoprotein Insulin Resistance Index (LP-IR) is a novel proprietary non-gender specific calculation for insulin resistance based on lipoprotein sub-particle size distribution. LP-IR is described as a reliable biomarker for progression to diabetes that reflects improvements in metabolic syndrome following dietary/lifestyle interventions with weight loss.
Objective: To compare post-diet/lifestyle intervention subjects who lost weight and decreased versus increased their LP-IR index.
Methods: Overweight/obese subjects with cardiovascular disease (CVD) or significant CVD risk factors enrolled in a 1 year intensive lifestyle intervention program including low fat (<10%) vegan diet. Risk factors, anthropometrics and biomarkers (including LP-IR, lipid profiles, etc.) associated with CVD risk were measured before and 1 year after intervention for comparison to weight loss changes. Subjects, stratified by LP-IR decrease or increase after 1 year, were compared using Wilcoxon nonparametric tests.
Results: Most participants (n=102, 49 males, 53 females) completed the program with weight-loss. Two groups were identified by LP-IR change: LP-IR score increase (25/102=24.5%); LP-IR decrease (77/102=75.5%). At baseline, there were no significant differences between these two LP-IR groups by age, BMI, systolic/diastolic BP, HDL/LDL/total cholesterol or triglycerides but mean LP-IR scores were significantly different (p=0.0019). Change in HDL-C, triglycerides, and LP-IR score after 1 year differed significantly between groups (p=0.0154, p=0.0024 and p=<0.0001, respectively).

	LP-IR Increased (N=25)			LP-IR Decreased (N=77)			Between Groups
Risk Factor	Baseline (SD)	Year 1 (SD)	% Change	Baseline (SD)	Year 1 (SD)	% Change	P-Value
BMI (kg/m2)	33.556 (7.715)	30.02 (7.409)	-10.54%	33.82 (6.73)	30.561 (6.225)	-9.64%	0.4435
Systolic BP (mm Hg)	134.16 (15.22)	126.56 (14.669)	-5.66%	137.143 (17.945)	128.182 (17.357)	-6.53%	0.7851
HDL-C (mg/dl)	48.32 (11.131)	43.96 (9.176)	-9.02%[b]	44.532 (13.5)	43.688 (11.679)	-1.90%	**0.0154**
LDL-C (mg/dl)	120.375 (32.87)	108 (29.376)	-10.28%	109.613 (39.539)	106.133 (34.511)	-3.17%	0.0555
T-CHOL (mg/dl)	206.92 (39.841)	193.8 (38.636)	-6.34%	191.26 (46.04)	179.013 (41)	-6.40%	0.7088
TG (mg/dl)	183.08 (110.671)	206.6 (116.635)	12.85%	183.701 (91.098)	147.701 (78.629)	-19.60%	**0.0024**
LP-IR	58.24 (20.001)	66.72 (21.384)	14.56%	71.662 (16.126)	55.714 (18.773)	-22.25%	**<.0001**

Conclusion: The majority of individuals who lose weight reduce their LP-IR. However, a subgroup (25%) of patients increased their LP-IR despite weight loss. The clinical and prognostic significance of these observations require further study.

Abstract Submitted for Poster Presentations:

- Kashani M, Eliasson A, Engler R, Turner E, Tschiltz N, Grunewald M, Halsey J, Fuller C, Villines T, Vernalis M. Prediabetes Reversal Using a Novel Comprehensive Health Model. *American College of Cardiology, 64th Annual Scientific Session*, March 14-16, 2015, San Diego, CA.

Abstract

Introduction: Over half of prediabetics will develop frank diabetes. Prediabetes is a modifiable risk factor for cardiovascular disease (CVD) warranting preventive intervention.

Objective: We examined the impact of a multicomponent intervention on the CVD risk profile of subjects with prediabetes who successfully reversed their disease without emphasizing weight loss.

Methods: Consecutive subjects of the Integrative Cardiac Health Project Registry, a 12-month CVD Risk Reduction Program focusing on four pillars: nutrition, exercise, stress and sleep improvement, completed validated questionnaires and were categorized as prediabetic (glucose Ó 100 mg/dL and < 140 mg/dL) or reverting prediabetes (glucose < 100 mg/dL). Diabetics were excluded from the analysis. Differences were analyzed using t-test.

Results: Of 508 subjects (56% women, mean age 53 Ñ13.5 years, 61% White, 22% Black, 5% Hispanic), 107 (21%) had prediabetes with mean HgA1C 5.9% and mean glucose 108.1 mg/dL. Of prediabetics, 52 (49%) reverted to normal glucose levels.

Risk Factor (n=52)	**Baseline**	**6-month**	**p value**
Fasting Glucose (mg/dL)	105.4 Ñ6.2	92.4 Ñ5.4	<0.001

Fasting Insulin (ulU/mL)	14.5 Ñ10.1	10.4 Ñ7.3	0.02
Homeostatic Model Assessment	3.8 Ñ2.7	2.4 Ñ1.7	0.002
Total Cholesterol (mg/dL)	190.7 Ñ41.1	175.1 Ñ39.0	0.05
Low Density Lipoprotein (mg/dL)	115.8 Ñ36.3	102.5 Ñ34.7	0.06
Systolic Blood Pressure (mm Hg)	134.3 Ñ15.5	127.9 Ñ13.1	0.03
BMI (kg/m^2)	30.0 Ñ5.7	29.0 Ñ5.8	0.40
Mediterranean Diet Questionnaire (14 points)	6.8 Ñ2.4	9.2 Ñ2.0	0.002
Aerobic Exercise Time (min/week)	136.4 Ñ139.1	192.9 Ñ161.7	0.05
Perceived Stress Scale (56 points)	21.9 Ñ7.4	18.7 Ñ7.0	0.03
Pittsburg Sleep Quality Index (21 points)	7.0 Ñ3.4	5.7 Ñ3.7	0.08
Fatigue Score (10 points)	4.2 Ñ1.9	3.3 Ñ2.0	0.03

Conclusion: A comprehensive health program emphasizing combined improvements in nutrition, exercise, stress and sleep may help subjects with prediabetes revert to normal glucose metabolism without substantial changes in BMI. Combatting progression to diabetes with a practical lifestyle intervention lowers CVD risk and improves overall health in this vulnerable population.

Abstract Submitted for Poster Presentations:

- Kashani M, Eliasson A, Engler R, Fuller C, Villines T, Vernalis M. Modest Elevation in Blood Pressure is a Red Flag for Cardiovascular Disease Risk. *AHA Epi/Lifestyle 2015*, March 2015.

Abstract

Introduction: Currently, cardiovascular disease (CVD) risk may be underestimated in subjects with modest elevation in BP as providers are advised to focus on the treatment of hypertension when managing blood pressure.

Objective: We examined the CVD risk profile of subjects with modest BP elevation to determine the prevalence of risk factors and to identify targets for intervention.

Methods: Consecutive subjects entering the Integrative Cardiac Health Project Registry (a 12-month CVD Risk Reduction Program) were assessed for anthropometrics and a CVD-relevant lab panel. Subjects were categorized as BP not elevated (≤120/80), modest elevation in BP (>120/80 and <140/90) and hypertensive (BP≥140/90). Comparisons were made between subjects with no BP elevation, modest BP elevation and hypertensives for differences in CVD risk factors using t-test.

Results: Of 352 subjects (56% women, mean age 53 Ñ13.5 years, 61% white, 22% black, 5% Hispanic), 114 (32%) had no elevation in BP, 154 (44%) had modest elevation in BP and 84 (24%) were hypertensive.

BP Category	BP mmHg	Glucose mg/dL	HOMA	HbA1C %	LDL mg/dL	HDL mg/dL	TG mg/dL	BMI kg/m^2	WC cm
Not Elevated	112/72	93.9 Ñ16.7	2.89 Ñ2.6	5.5 Ñ0.6	108.5 Ñ28.7	60.4 Ñ17.0	97.6 Ñ50.7	28.2 Ñ5.8	94.3 Ñ15.1
Modestly Elevated	128/80	100.6 Ñ14.9	3.75 Ñ3.8	5.7 Ñ0.6	115.0 Ñ38.0	55.2 Ñ13.6	115.7 Ñ66.1	30.5 Ñ5.5	102.8 Ñ14.1
p value		0.001	0.01	0.02	0.12	0.009	0.012	0.0006	0.0001

BP = blood pressure, HOMA = homeostatic model assessment, HbA1C = hemoglobin A1C, BMI = body mass index, WC = waist circumference.

For the parameters above, there were no differences between the hypertensive group and the group with modest elevation in BP.
Conclusion: CVD risk factors appear to be increased in subjects with modest elevation in BP, placing these subjects at increased risk for CVD much like subjects with hypertension. Specifically, dyslipidemia, glucose dysmetabolism and obesity co-exist with very modest increases in BP, laying a foundation for metabolic syndrome. In applying current BP guidelines to this population of predominantly middle-aged women, subjects would be left vulnerable and unrecognized for their increased risk. Such subjects deserve close scrutiny for their comorbid risk factors and interventions for aggressive management.

The following are key activities accomplished in the past year:

- Strategic plan developed to synchronize clinical approach to comprehensive prevention and CV health by standardizing practices of 3 ICHP Nurse Practitioners in interacting with patients and clinical team.
- ICHP clinical guidelines updated to reflect latest evidence of cardiovascular health practice in preparation for initiation of research protocols.
- Development and implementation of Executive Medicine Program at request of OTSG:
  - Request from Office of the Surgeon General (OTSG) to learn more about ICHP program and its potential in impacting the health and wellness of General Officers. Informational meeting with nutritional food demonstration conducted on 12/15/13 with very positive feedback from OTSG.
  - Creation of customized program to address health of our nationôs leaders. Program involved numerous meetings for strategic planning and development of outreach IT software application for collection of health surveys. Multidisciplinary staff expertise used to create personalized plans of care, based on the ICHP model, for spouses of four-star generals and the Army Surgeon General (TSG).
  - Highly successful event (March 4, 2014) executed at the TSGôs home at Ft. McNair conducting an interactive Healthy Living Workshop customized for leaders in highly stressed occupations. TSG has requested to collaborate further with the ICHP team based on the success of the March event.
  - 2nd Executive Medicine program conducted July 2014 at ICHP with new VIP cohort. BG Clark, WRNMMC Hospital Commander, addressed this cohort.
- ICHP Database and Platform Creation continued:
  - Provider specific meetings with IT specialists conducted for clinical feedback
  - Front desk process and flow developed to dovetail with clinical milestones. Review and refinement of previous edits completed in anticipation of beta testing projected for November.
  - Front end-survey mechanism built with clinical end pending testing.
- Data Management Plan in progress with merging of data from two previous databases in order to move forward with one dataset for further analysis.
- Updated the standardized approach to Carotid Intimal Media Thickness measurement and interpretation for research protocols with utilization of new equipment.
- Standardized Coaching Call Process refined and implemented to encourage adherence to lifestyle changes in order to maintain gains.
- ICHP Stress Management CD in development for production by Dr. Gordon in WRNMMCôs behavioral health service to include tracks: 1) Rise to a New Day 2) Tension Tamer 3) Power

Down for Restful Sleep.

- ICHP based cognitive behavioral therapy (CBT) intervention for insomnia utilizing CME developed and submitted for WRNMMC IRB review.
- Adoption of ICHPôs Lifestyle Prescriptions by the Army Surgeon General (TSG) for BG Clarkôs Resiliency efforts at WRNMMC. ICHP is honored to provide this service.
- Collaborative efforts to expand scientific molecular work with clinical centers of excellence.
- Future protocols to emphasize unified ICHP approach to combat CVD, cognitive decline and cancer.

Sub Task #3.1 Continuation of the "Validation of the ICHP Cardiovascular Risk Score" protocol.

Methodology

Data previously collected on patients enrolled in the Prospective Army Coronary Calcium (PACC) and PACC Rescan projects were reviewed. Specific information was gathered and analyzed to give each patient a CV disease risk score according to a formula developed by the ICHP. This ICHP formula uses the Framingham model of risk prediction and adds historical factors and biochemical markers to produce a novel score predictive of CV disease risk in military beneficiaries. The goal of the study was to validate the utility of this novel ICHP scoring system by comparing the predicted risk with outcomes in this well characterized population. The primary objective of the project was to validate the predictive utility and accuracy of the ICHP CV risk score (or ICHP score). Specifically, the goals: a) to determine if the ICHP score correlates with cross-sectional prevalence of coronary calcium as measured in the PACC project and b) with the development of CHD events such as angina, myocardial infarction, or need for CV intervention such as coronary stenting, angioplasty, or bypass surgery. A third goal: c) to determine the correlation of the ICHP score with coronary calcium progression as measured in the PACC rescan project.

Key Findings/Conclusions:

Key study findings were most recently reported in the W81XWH-11-2-0227 (FY12-14 Yr 1) Annual Report dated October 27, 2012.

Status:

Manuscripts Published (See Appendix A):

- Kashani M, Eliasson A, Bailey K, Vernalis M. A systematic approach incorporating family history improves identification of cardiovascular disease risk. *J of Cardiovasc Nurs*. 2014 May 20. [Epub ahead of print]

The following are key activities accomplished during the past year:

- ICHP Clinical Decision Support Tool actively applied to clinical encounters to improve CVD risk classification.
- Inclusion of new cardiovascular risk scoring systems (i.e. 10-year risk and lifetime risk) in our clinical model given the guidance from the American College of Cardiology Conference 2014.
- Implemented the ICHP Clinical Decision Support Tool used in clinical encounters to improve CVD risk classification.
- Analysis of data to look at decreasing CVD and increasing self-efficacy scores for

publication.
- Initiatives to capture ICHPôs impact on health *improvement* as well as CVD risk reduction: Quantitative approaches to LIFE Score (Life Impact for Empowerment)

Sub Task #3.2: Initiate the "ZENITH (randomiZed Evaluation of a Novel comprehensIve prevention program on aTHerosclerosis progression) Trial".

Methodology

The purpose of this one-year, prospective, randomized, controlled, interventional trial is to investigate the impact of ICHP-CPP on vascular health, atherosclerosis progression and left-ventricular relaxation (diastolic function) among patients with increased lifetime CVD risk, but low short term coronary heart disease (CHD) risk (according to the Framingham Risk Score, FRS) as compared to receiving usual care (UC). Up to 170 male and female patients between 18-50 years of age with low (<10%) 10-year FRS for CHD but estimated lifetime risk (to age 95 years) of coronary death or myocardial infarction (MI) of Ó39% without clinically manifest CVD [MI, coronary or peripheral arterial revascularization, obstructive coronary artery disease (CAD), heart failure or cerebrovascular event] will be randomized to participation in the currently ongoing ICHP-CPP or to UC. The primary endpoint is between-group differences in the change in vascular endothelial function as measured using DTM, as reported as adjusted. Secondary endpoints are changes in measures for CIMT, cardiac diastolic function, lifetime CHD risk scores, and the ICHP CV Risk Score. It is hypothesized that patients with low-short term (Framingham 10-year CHD risk score) but high lifetime estimated risk for coronary death or MI who participate in the ICHP-CPP will improve vascular health and reduce atherosclerosis progression when compared to those receiving usual care.

Status:

Annual CR/addendum submitted to WRNMMC IRB on 30 Oct 13 and approval received 7 Jan 2014; forwarded to HRPO. Addendum updates recruitment materials, case report forms, change in temporary serum storage location at WRNMMC and several edits to investigator information. WRI protocol submitted to Chesapeake IRB on 6 Mar 14; determination was made that this study is non-human subject research at WRI. Replacement carotid ultrasound equipment was received and training completed. Collection of echocardiogram images resolved and collection protocol in place. Transfer and archiving of echocardiogram research images is in the final stages of resolution; actively working with WRNMMC Clinical Informatics Department to resolve. Recruitment commenced early July. Recruitment has been slow; plan submission of protocol addendum with next CR to extend recruitment plan to public spaces and additional WRNMMC clinical areas. Eight patients have been screened to date; 3 meet criteria to enroll, but all wanted participation in non-study ICHP-CHP program.

Sub Task #3.3: Initiate the "Cardiovascular Prevention Program (CPP) Registry for the Integrative Cardiac Health Project" protocol.

Methodology:

The purpose of this study is to establish a registry to enable research on patients at risk for cardiovascular disease (CVD). All clinically derived patient-related data for subjects participating in the WRNMMC CHP will be entered into a single, secure database. At periodical intervals, assessment of the registry database will allow queries to define the impact of an integrative lifestyle change program on CVD risk over time. The ICHP Registry will utilize the

ICHP database which documents demographics, responses to validated lifestyle habits questionnaires regarding exercise, diet, stress and sleep, physical examination and anthropometrics, laboratory test results, imaging, actigraphic data, clinical recommendations and consultations, participant management, and participant visits.

Patients will be offered enrollment into this study at the time of presentation if they are military health care beneficiaries and are at least 18 years of age. All participants, regardless of enrollment in the study, will receive the usual standard of care by their health care providers. Collection of medical information on ICHP subjects is accomplished through interview of patients as well as through review of medical information from other facilities providing care. Clinical data collection occurs at baseline and at the conclusion of the intervention, typically at 6 months. Additional follow up for support of the patientôs gains and additional data collection occur at 12 months and annually for up to 5 years. The research component of this study will involve the analysis of clinical data collected at these intervals.

The ICHP clinical database can be queried at a single sitting with removal of all personally identifying information to perform assessments of prevalence of risks, associations of behaviors and risks, and the success of various interventions over time. Such queries take minutes to perform and can be accomplished with minimal risk to individual privacy. There is no need to maintain any linkage data as the information is harvested at a single sitting from one database requiring no marriage with external data sets.

Status:
Protocol approved by WRNMMC IRB on 27 Mar 13; HRPO approved on 13 Nov 13. WRNMMC CR/amendment approved on 27 Mar 14; amendment updates survey tools currently being used in ICHP CHP. Recruitment delayed until initial ICHP database testing is complete. An addendum to further clarify follow-up portion of the ICHP lifestyle program is planned next quarter.

Sub Task #3.4 Collaboration on "Assessing Risk Factors for Cardiovascular Disease in Individuals with Traumatic Amputations" protocol (PI: Alison Pruziner), DPT, ATC, WRNMMC Dept of Rehab).

Methodology:

The objective of this comparative cohort study is to assess presence of known risk factors for CVD in individuals with traumatic amputations. Up to 405 participants will be enrolled and divided into three groups: no injury, traumatic orthopedic injury with amputation, traumatic orthopedic without amputation. Data will be collected at two time points, at time of consent and at a 5-year follow-up visit, and will include demographic (including diagnosis of hypertension, hyperlipidemia or diabetes mellitus) and family history, anthropometric (height, weight, waist circumference, hip circumference and body composition), biochemical (lipids, fasting blood sugar, hemoglobin A_{1c}, fasting insulin, ultra-sensitive C - reactive protein, lipoprotein (a), thyroid stimulating hormone, vitamin D, and fibrin D-dimer), blood pressure, heart rate, pulse pressure, EKG, carotid intima-medial thickness (CIMT) study, stress and sleep surveys, diet (fruit and vegetable intake, total fat and saturated fat intake), smoking history and activity measures. CVD risk will be estimated using the Integrated Cardiac Health Project (ICHP) risk assessment and the National Heart Lung and Blood Institute (NHLBI) 10-year risk estimate. It is hypothesize that: 1) Individuals with traumatic amputations (A)

will have higher levels of factors that increase risk (anthropometry, biochemical markers, blood pressure, pulse pressure, CIMT, stress, poor sleep habits, saturated fat intake, smoking) and lower levels of factors that decrease risk (fruit and vegetable intake and activity) for CVD when compared to individuals without orthopedic injuries (N), and that this risk will continue to increase over the 5-year follow-up; 2) Individuals with traumatic amputations (A) will also have the same increased risk factors, as stated above, when compared to individuals with traumatic orthopedic injuries that did not result in amputation (O), and again this risk will continue to increase over the 5-year follow-up, and; 3) There will be no difference in presence of risk factors between individuals with (O) and without orthopedic injuries (N), that did not result in amputation.

Status:
Total study enrollment=52 (24 controls, 25 amputees, 3 limb salvage): 4 new patients enrolled in past year. Enrollment delays are due to limited recruitment support for PI, inability to perform carotid ultrasound and plans to collect serum for biomolecular work. Addendum submitted to WRNMMC DRP on 11 Oct 13 for collection of additional blood samples for molecular analysis along with PI change to Dr. Alison Linberg approved on 10 Feb 14; approved by USAMRMC ORP on 14 Apr 14. A recent amendment for PI name change from Linberg to Pruziner and change in processing of MAP biomarker sample submitted to WRNMMC DRP approved July 28, 2014; forwarded to MRMC ORP. Staff identified from Department of Rehabilitation to assist in patient recruitment and processing of blood sample for biomolecular work. Training was conducted in WRNMMC Biomolecular Research Lab (BRL) in collaboration with Windber Research Institute on processing of these blood samples.

Task #4: Follow-up data analysis and publications for the following protocols at WRI: 1) Global Profiling of Gene/Protein Expression and Single Nucleotide Polymorphisms Associated with Coronary Heart Disease Reversal and the Sub-Study for Subjects in the Dr. Dean Ornish Program and 2) Cardiovascular Risk Assessment and Prevention Program through the Cardiovascular Risk Clinic (CRC).

Methodology

Follow-up data analysis and publications for the following protocols at WRI:
1) Global Profiling of Gene/Protein Expression and Single Nucleotide Polymorphisms Associated with Coronary Heart Disease Reversal and the Sub-Study for Subjects in the Dr. Dean Ornish Program and 2) Cardiovascular Risk Assessment and Prevention Program through the Cardiovascular Risk Clinic (CRC). Although enrollment in these programs is complete, we will continue to finalize data collection as well as conduct data analysis on biochemical markers, gene expression and SNP data, and PET/CT imaging studies and will prepare results for publication.

1) Global Profiling of Gene/Protein Expression and Single Nucleotide Polymorphisms Associated with Coronary Heart Disease Reversal and the Sub-Study for Subjects in the Dr. Dean Ornish Program

1a) Ornish Program

Status:
Enrollment into the Dr. Dean Ornish Program is closed and all active participants have completed their participation in the study. Data analysis is ongoing.

Subject Enrollment and Demographics

The Ornish program is closed to enrollment and all active subjects have completed the program. Subject enrollment was 422 participants including 25 cohorts and 4 retreats. 339 participants graduated from the program and 83 participants discontinued participation (20% dropout rate). Demographic characteristics of participants were: average age of 66.1 years, 53% female, 33% veterans or the spouse of a veteran, and 41% had diagnosed coronary heart disease.

Outcome Data

Participants in the Dr. Dean Ornish Program at Windber Medical Center achieved significant improvement in levels of virtually all of the measured coronary artery disease (CAD) risk factors over the initial 12-week period. Measures of obesity including weight and BMI declined ~7%, levels of total cholesterol were reduced by nearly 13%, blood pressure dropped ~9%, measures of physical fitness increased more than 26%, and levels of depression decreased approximately 47%. These data demonstrate that lifestyle change programs may be important for primary prevention in individuals with diagnosed CAD and those at increased risk of disease. Over the course of one year, weight and BMI decreased ~9%, diastolic blood pressure decreased ~7%, measures of physical fitness increased 25%, and levels of depression decreased nearly 50%.

1b) Global Profiling Status:

Enrollment to the global profiling study is closed and all active participants have completed their participation in the study. Enrollment in the sub-study was closed as of July 27, 2007. Data analysis is ongoing.

Subject Enrollment and Demographics

Subject enrollment was 374. There were 166 participants taking part in the lifestyle change program, 140 subjects serving as the control group, and 68 participants enrolled in the Sub-study. Demographic characteristics of the control group were: average age of 63.7 years, 51% were female, 29% were veterans or the spouse of a veteran, and 34% had diagnosed CHD.

Data:

Lipoproteins ï LipoScience devised the new LP3 analysis process to better account for the full diversity of plasma lipoproteins that span a continuum of particle diameters and a Lipoprotein Insulin Resistance Score (LP-IR), which is significantly associated with insulin resistance. Using previously collected data in the Ornish and CRC programs, the following abstract was prepared and submitted to the American Cardiology of Cardiology:

Abstract submitted:

- Ellsworth DL, Mamula KA, Blackburn HL, Engler RJM, Vernalis MN. Cardiac lifestyle interventions differing in dietary stringency improve insulin resistance through changes in lipoprotein profiles. *American College of Cardiology, 64th Annual Scientific Session*, March 14-16, 2015, San Diego, CA.

Abstract

Background: Metabolic dysfunction characterized by insulin resistance (IR) is an important risk factor for developing type-2 diabetes and coronary artery disease (CAD). The Lipoprotein Insulin Resistance (LP-IR) score, derived from measures of lipoprotein subclass particle

concentration and size, is a new measure for assessing IR and identifying patients with increased risk for developing diabetes. Lifestyle modification interventions are known to mediate CAD risk through traditional measures such as blood pressure, lipids, and BMI; however, the effects of dietary stringency on IR and molecular drivers of the LP-IR score are unclear.
Methods: Patients with CAD or significant CAD risk factors participated in 1 of 2 clinical lifestyle interventions differing in dietary stringency: 1) an intensive non-randomized program with a strict vegetarian diet (n=90 subjects with 90 matched controls) and 2) a moderate randomized trial following a Mediterranean-style diet (n=90 participants, 58 controls). Changes over 1 year in lipoprotein profiles, LP-IR score, and traditional CAD risk factors were assessed by Wilcoxon Signed Rank tests.
Results: Participants in the intensive lifestyle intervention had poorer baseline cardiovascular health (significantly higher BMI, total cholesterol, triglycerides, LP-IR) than patients in the moderate program. Both interventions led to weight loss (-8.9%, intensive program; -2.6%, moderate program; P<0.001) and a significant decrease in LP-IR score (-13.3%, intensive; -7.9%, moderate; P<0.01) compared to respective controls over one year. Of the six lipoprotein parameters that comprise the LP-IR score, only large VLDL/chylomicrons decreased significantly in patients compared to controls in both programs (-26.3%, intensive; -13.7%, moderate; P<0.05).
Conclusions: Lifestyle modification including a Mediterranean diet is comparable to a stringent intervention with a vegetarian diet for improving insulin resistance defined by LP-IR. Significant reductions in large VLDL/chylomicrons may drive improvement in IR irrespective of dietary stringency.

Macrophage migration inhibitory factor (MIF) ï MIF is an inflammatory cytokine that regulates smooth muscle cell migration and proliferation, and thus plays an important role in promoting development of atherosclerotic lesions. MIF has been shown to be an important biomarker for diseases with inflammation, such as CVD, diabetes, obesity, and cancer. A draft manuscript summarizing results has been prepared and additional revisions are ongoing.

Gene Expression ï The manuscript comparing changes in gene expression in Ornish vs Control participants was published in *Circulation: Cardiovascular Genetics*. The reference is provided below:

Manuscript published (See Appendix A):

- Ellsworth DL, Croft DT Jr, Weyandt J, Sturtz LA, Blackburn HL, Burke A, Haberkorn MJ, McDyer FA, Jellema GL, van Laar R, Mamula KA, Vernalis MN. Intensive cardiovascular risk reduction induces sustainable changes in expression of genes and pathways important to vascular function. Circ Cardiovasc Genet 2014;7:151-160.

Additional analyses of the gene expression data have been conducted with patients stratified by weight loss. A manuscript has been prepared and will be submitted to the journal *Obesity* during the next quarter. The abstract and reference of the paper are provided below:

Manuscript in preparation:

- Ellsworth DL, Mamula KA, Blackburn HL, McDyer FA, Jellema GL, van Laar R, Engler RJ, Vernalis MN. Importance of substantial weight loss for altering gene expression during

intensive cardiovascular lifestyle modification. *Obesity* (to be submitted).

Abstract

Objectives: To examine relationships between weight loss through changes in lifestyle and leukocyte gene expression profiles.
Methods: A prospective nonrandomized trail was conducted over 1 year in participants undergoing intensive lifestyle modification to reverse or stabilize progression of coronary artery disease. Cardiovascular risk factors, inflammatory biomarkers, and peripheral blood gene expression as a function of weight loss were assessed in 89 lifestyle participants and 71 retrospectively matched controls undergoing usual care.
Results: Substantial weight loss (-15.2±3.8%) in lifestyle participants (n=33) was associated with improvement in selected cardiovascular risk factors and significant changes in leukocyte gene expression from pre- to post-intervention. Approximately 10% of the transcriptome (2225 unique genes) showed significant expression changes at a false discovery rate corrected *P*-value <0.05. Altered molecular pathways were related to immune function and inflammatory responses involving endothelial activation. In contrast, participants losing minimal weight (-3.1±2.5%, n=32) showed only minor changes in cardiovascular risk factors, markers of inflammation, and gene expression compared to non-intervention controls after 1 year.
Conclusions: Widespread gene expression changes associated with vascular immune and inflammatory responses were associated with substantial but not minimal weight loss during intensive lifestyle modification for cardiovascular risk reduction.

Structural and Functional Measures of Cardiovascular Health ï Specific endpoints measured include ejection fraction and wall motion, coronary artery calcification scores, left and right ventricular volumes, myocardial mass, stenosis sizing and vessel diameter, plaque density and differentiation of calcified versus non-calcified plaque, and tissue perfusion and viability. PET/CT scanning analysis continues; collaboration ongoing with Dr. Edward Miller, Boston University to provide clinical insight into data. Results of the initial analysis indicate that many ECT variables were significantly different between cases and controls at baseline, and that few measures showed significantly different changes between groups. Analysis is ongoing.

SNP Variation and Obesity ï During the year, 155 samples from intensive lifestyle participants were genotyped for the following obesity-related SNPs: rs9939609, rs1421085, rs17782313, rs2815752, rs7498665, rs142433, rs925946, rs2116830, rs2241423, rs12444979, rs6548238.

Dropouts ï A manuscript describing reasons why participants discontinue participation in lifestyle modification programs was completed. The paper was rewritten from the original version to focus only on the Ornish program and to examine gender differences in attrition. The reference and abstract for the paper are provided below. The paper will be submitted for publication during the next quarter.

Manuscript in preparation:

- Mamula KA, Vernalis MN, Ellsworth DL. Attrition from lifestyle modification programs for cardiovascular risk reduction: gender specific considerations and predictors. (to be submitted).

Abstract

Identifying significant predictors of attrition from lifestyle modification programs is central to improving treatments for cardiovascular disease. To address this issue, we conducted a retrospective observational study of women (n=178) and men (n=160) who enrolled in a clinical lifestyle intervention designed to stabilize or reverse progression of heart disease through changes in lifestyle. Pretreatment (baseline) and initial treatment-related variables were examined separately in women and men to assess utility in discriminating eventual dropouts from completers. Gender specific stepwise regression models contained three common variables: body mass index at entry, dietary compliance, and education level, but neither model accurately predicted attrition. Self-reported reasons for dropping out differed between women and men: noncompliance with the program guidelines and medical/health problems were important issues for women, while work-related conflicts were most prevalent in men. Clinical trials and lifestyle programs for cardiovascular risk reduction should recognize that personal barriers to continued participation differ between women and men and must strive to accommodate all barriers in order to maximize patient retention. Trial registration (ClinicalTrials.gov): NCT01805492

Dietary Analysis ï The original objective of this project was to assess changes in dietary components during the CRC and Ornish programs. We recently expanded the focus to include vitamin supplements. During the year, dietary data were entered electronically and analyzed by Food Processor 10.10 software program for 85 participants at 3 time points each (255 questionnaires). Food Processor results were entered into the Access database. Analysis of dietary changes showed the following: (1) significant changes in total calories (-13%), % calories from fat (-56%), and % calories from carbohydrates (+28%) during intensive lifestyle change; (2) significant changes in the following dietary components: calories from saturated fat (-75%), dietary fiber (+61%), mono-unsaturated fat (-70%), poly-saturated fat (-39%), trans fats (-76%), cholesterol (-87%); (3) significant changes in vitamins: A (+39%), B6 (+29%), C (+38%), K (+97%). A low fat diet provides more minerals than a high-fat diet. Comparisons with the CRC program are in progress.

An analysis of dietary trace elements showed: iron (+41% change, P=0.001), zinc (+5% change, P=0.311), copper (+30% change, P=0.087), manganese (+99% change, P<0.001), chromium (+14% change, P=0.796), molybdenum (+15% change, P=0.878) during lifestyle modification. Data entry for vitamin intake at baseline, 12 weeks, and 1 year is ongoing.

2) Cardiovascular Risk Assessment and Prevention Program through the Cardiovascular Risk Clinic (CRC)

Methodology:

This program was established as a platform to address the unique needs of retired military beneficiaries at risk for CV disease. The program mirrors the Cardiac Prevention Program (CPP) designed and established by the ICHP at WRAMC. It includes conventional and novel CV risk profiling and tailored, personalized behavioral recommendations for primary or secondary prevention by an integrative team of providers comprised of a nurse case manager, psychologist, nurse practitioners, dietitians, stress management instructors, and exercise physiologists. Validated tools to screen for and measure CV risk, stress, sleep health, compliance with dietary recommendations and exercise are standard of care. The program is an adjunct to the best medical practices provided by their primary care provider.

Phase I of the program involves each participant undergoing a comprehensive health risk assessment that is completed by a physician, followed by a four- hour ñPearls for your Heartò workshop and participants then schedule individual appointments with each modality specialist to receive education and counseling in nutrition, exercise, stress management and mind/body health. These are monthly appointments to be completed over a 4-6 month period.

Phase II of the program begins after the completion of the healthy lifestyle intervention (Phase I). During this phase each participant will again meet with the physician. During this appointment the physician will prepare the participants for the next phase and give them strategies for maintaining success on their own. The second phase of the Program provides additional reinforcement through monthly phone calls with an integrative health coach. Participants will remain in Phase 2 for five years, during which time they will come to the center for re-assessments every six months.

Status:
Enrollment in this program ended June 30, 2013; data analysis is ongoing.
Subject Enrollment and Demographics:
Demographic characteristics of participants were: average age 58.9 years, 58% female, 22% veterans or the spouse of a veteran, and 20% with diagnosed coronary heart disease. Total subject enrollment was 264 (144 intervention; 120 controls); 59 drop-outs; 34 control participants transferred to the intervention arm after one year as a control. A summary of final patient progress is as follows:
- 124 intervention participants completed the intervention (4-6 months); 102 completed first 6^{th} month follow-up time point; 68 completed year 1; 45 reached month 18; 26 reached year 2; 14 completed Month 30; 11 have completed year 3.
- 91 controls completed the ñwaiting period completeò time point (6 months); 66 completed the first 6^{th} month follow-up time point; 29 reached year 1; 21 completed month 18; 11 reached year 2; 8 have completed year 3.
- 90 intervention participant satisfaction surveys were completed and returned

A batch of 15 samples for plasma markers leptin, insulin, hsCRP, and glucose; 13 samples for serum markers adiponectin, serum amyloid A, vitamin D, and lp(a) including a universal control sample were sent to LipoScience and Johns Hopkins.

Gene Expression ï The RNA from 59 CRC blood samples was isolated from PAXgene tubes, globin cleared, amplified, fragmented, and scanned on U133A 2.0 expression arrays. Call rates were 50.3-62.4%. An additional 32 CRC samples have been isolated and globin cleared. RNA concentrations ranged from 32.8-198.5 ng/µl; OD260/280 ratios were 2.08-2.29; RIN numbers were 7.8-9.2.
DMET arrays were run on DNA samples from 40 CRC participants; call rates were 98.24-99.90%. Thirty-one CRC samples were genotyped using two TaqMan SNP assays: rs12444929 and rs1421085.

SNP variation and triglycerides ï Genotyping of 117 CRC participants completed for 19 SNPs potentially associated with triglyceride response. Statistical analysis is ongoing.

Task #6: Initiate "Exploring the Predictive Patterns of the Natural History of Pre-diabetes: Proof of Principle Study" protocol at WRNMMC (PI – COL Robert Vigersky, Diabetes Institute).

Methodology

The primary purpose of this prospective, observational, proof of principle study is to determine the feasibility of using a novel, point-of-care (i.e. home), multiple analyte test platform (Theranos) to study the temporal changes in five biomarkers related to glucose dysregulation, inflammation, vascular dysfunction, and immunity that can lead to diabetes and increased cardiovascular risk [insulin, leptin, high sensitivity Troponin T (hs-cTnT), high sensitivity C-reactive protein (hs-CRP), and ferritin]. A secondary purpose is to examine patterns of gene expression in peripheral blood in patients diagnosed with pre-diabetes who are entering into an intensive lifestyle modification program.

Up to 50 adult military healthcare beneficiaries ($\geq$ 18 years) who met the screening criteria for pre-diabetes and have self-referred or been referred to the ICHP-CPP for CV risk reduction will be enrolled. Each participant will be provided a portable, home-based Theranos system and be asked to provide a fingerstick (FS) blood sample to the system at three specific times per week for 2 months pre-initiation and for the duration of their participation in the lifestyle change program.
Blood samples will be collected prior to (2 months) and at the conclusion of the lifestyle program (8 months) to evaluate changes in gene expression and to determine changes in the biomarkers noted above. Blood samples will be collected again at 12, 24, and 36 months to determine if there are additional changes in the genetic markers and if the biomarkers are a measure of their dysglycemia.

A variety of statistical techniques will be used, depending on the level of measurement of the variables we are modeling (e.g., binomial, multinomial, continuous) to characterize the dynamic relationship between the analytes obtained by the Theranos system and 1) metabolic and CV risk and 2) advancement to diabetes and/or CVD.

Status:

WRNMMC protocol submitted to DRP on 9 May 12 and IRB approval received on 6 Dec 12 with subsequent 2nd level approval by USAMRMC HRPO on 4 Jun 13. The WRI protocol was approved by the WMC IRB on 17 May 13 and by the USAMRMC HRPO on 24 July 2013. The WRI protocol was then submitted to the Chesapeake IRB on March 10, 2014, who determined that this study was non-human subject research at WRI. Sub award for Geneva Foundation executed and study planning has begun; awaiting execution of Theranos, Inc. contract. Plan amendment submission to clarify temporary blood storage location at WRNMMC and updated ICHP lifestyle program survey tools. Recruitment is pending.

Task #7: Continue study entitled "Metabolic and Biomolecular Biology Study Studies in Surgical Interventions for Morbid Obesity" as a component of the Integrative Cardiac Health Program at WRI.

Methodology

This study represents a collaboration involving WRI, Windber Surgery Center, and WRNMMC. The purpose of the study is to characterize (1) biomolecular profiles in adipose tissue at baseline that are predictive of significant differences among individuals in rates of future weight loss, and

(2) longitudinal biomolecular changes in peripheral blood that correlate with rates of weight loss in obese patients.

Status:

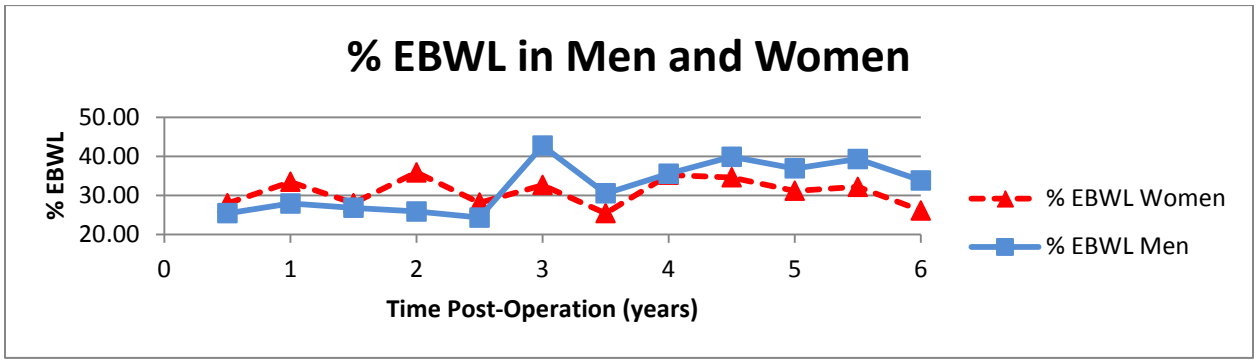
USAMRMC approval received 15 June 12; protocol approved by Chesapeake IRB on 25 Feb 14. To date, the total enrollment is 280; 247 active participants, 31 drop-outs; 72 have only 1 follow-up, 38 have 2 follow-ups, and 51 have 3 or more.

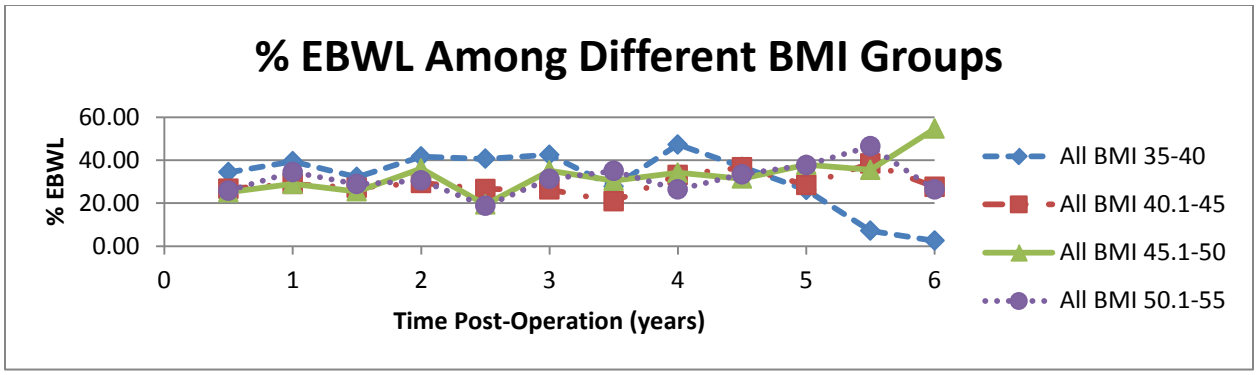
During the year, no new lap-band patients were consented to participate in the research study. Follow-up blood samples were obtained from 47 participants and 664 plasma/RBC aliquots were processed and stored; 112 plasma aliquots for each biomarker, which included a universal control, were sent to LipoScience for lipoprotein analysis and Johns Hopkins Bayview Medical Center for leptin, insulin, hsCRP, and glucose testing.

Blood RNA samples from 210 patients were globin-cleared, amplified, and run on gene expression arrays. Call rates on expression arrays were 51.7-62.5%. RNA concentrations were 21.1-218.5 ng/µl, OD260/280 ratios were 2.06-2.26; RINs were 7.0-9.1. We are waiting for microarrays to complete the expression analysis.

During the year, 73 samples from LAGB patients were genotyped for the following obesity-related SNPs: rs9939609, rs1421085, rs17782313, rs2815752, rs7498665, rs142433, rs925946, rs2116830, rs2241423, rs12444979, rs6548238. DNA was isolated from 45 blood samples; concentrations were 7.7-324.6 ng/ul, OD260/280 ratios were 1.89-2.27.

The percentage of excess body weight loss (%EBWL) was calculated. Trends in long-term weight loss were examined by random coefficients models and Kruskal-Wallis Rank-Sum Tests. Results show that there is no significant evidence (95% confidence level) that there are differences in %EBWL across time points for the group as a whole, other than that the overall group experiences positive %EBWL with respect to baseline. After 6 months, significant changes in %EBWL did not occur.





Comparison of lipoprotein changes in a surgical intervention vs lifestyle modification ï The following abstract, which compared lipoprotein changes following a surgical intervention vs lifestyle modification, was presented as a poster at the Obesity Society meeting:

Abstract presented as poster:

- Blackburn HL, Mamula KA, Haberkorn MJ, Burke A, Slavik JE, Sann NJ, Marley KR, Vernalis MN, Ellsworth DL. Differential effectiveness of laparoscopically-adjustable gastric banding versus lifestyle modification for modifying plasma lipoprotein profiles. *Obesity 2013: 31st Annual Scientific Meeting*, November 11-16, 2013, Atlanta, GA.

Abstract

Obesity is an important cardiovascular disease (CVD) risk factor implicated in dyslipidemia and vascular dysfunction. Although LDL lowering is often a primary goal of therapy, the size and concentration of lipoproteins provide additional information on the true atherogenicity of plasma lipids. Surgical and lifestyle interventions are options for weight loss, but little is known about their effects on lipoproteins.

Changes in BMI and plasma lipoproteins over 1 year were compared between 31 patients undergoing laparoscopically placed adjustable gastric banding (LAGB) and matched participants in 2 lifestyle change programs differing in scope and intensity. Lipoprotein profiles were determined by nuclear magnetic resonance (NMR) spectroscopy. Baseline values were compared using Wilcoxon Signed Rank tests for matched pairs; changes over time were assessed by paired t-tests.

Over 1 year, LAGB led to significantly lower BMI (-16%, $p<0.001$ vs baseline) than intensive (-8%, $p<0.001$) or moderate (-2%, $p<0.05$) lifestyle change (matched-pairs $p<0.001$ in both comparisons). Notably, lipoprotein responses differed between interventions. Intensive lifestyle led to clinical changes in total LDL particles (ï 10%, $p<0.05$ vs baseline and matched pairs), while LAGB resulted in a significant increase in HDL particles (+19%, $p<0.001$) versus intensive (+1%) or moderate (ï 6%, $p<0.05$) lifestyle change (matched pairs $p<0.001$).

LAGB surgery and lifestyle change led to weight loss and changes in lipoprotein subclasses; however, the interventions may affect CVD risk through different pathways. Lifestyle reduced the atherogenicity of LDL lipoproteins, which may inhibit inflammation and endothelial

dysfunction. Gastric surgery improved the number of HDL particles and may protect against CVD through anti-inflammatory and antioxidant activities.

Task #8: Initiate the "Global Profiling of Gene/Protein Expression and Single Nucleotide Polymorphisms Associated with Coronary Heart Disease Reversal: Long-term Follow-up Sub-study at WRI.

Methodology

Study is assessing long-term maintenance (3-7+ years) of selected physical parameters, psychometric measures, plasma lipids, and peripheral blood gene expression in past participants of the Ornish Program to understanding whether traditional risk factor and molecular changes persist over time and contribute to long-term risk reduction.

Status:

Three examinations were conducted for past participants; exams consisted of blood draw, psychometric surveys, standard anthropometric measurements, and 3-day dietary recall. Recruitment is complete; a total of 49 participants enrolled in the study.

For all 49 participants, collected and collated all data forms, completed all food diaries in Food Processor, scored psychosocial surveys, and entered all data including medication/vitamins and physical assessment forms into the database. 49 RNA samples from long-term study participants were isolated, globin cleared, amplified, and fragmented; concentrations were 18.9-125.8 ng/μl, OD260/280 ratios were 2.01-2.27; RINs were 7.8-9.0. All RNA samples were run on U133A 2.0 expression arrays with call rates >58.51%. Analysis of gene expression will be conducted during the next quarter.

References:

1. Shahriar S, Masumi M, Edjtehadi F, et al. Cardiovascular risk factors among males with war-related bilateral lower limb amputation. *Mil Med.* 2009;174(10):1108-11.

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3. Kones R. Molecular sources of residual cardiovascular risk, clinical signals, and innovative solutions: relationship with subclinical disease, undertreatment, and poor adherence: implications of new evidence upon optimizing cardiovascular patient outcomes. *Vasc Health Risk Manag*. 2013;9:617-70. Epub 2013 Oct 21. Review. Online at http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3808150

4. Kashani M, Eliasson A, Vernalis M, Costa L, Terhaar M. Improving assessment of cardiovascular disease risk by using family history: an integrative literature review. *J Cardiovasc Nurs*. 2013 Nov-Dec;28(6):E18-27. doi: 10.1097/JCN.0b013e318294b206.

5. Goff DC Jr, Lloyd-Jones DM, Bennett G, et al. 2013 ACC/AHA guideline on the assessment of cardiovascular risk: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines. *Circ.* 2014 Jun 24;129(25 Suppl 2):S49-73. Epub 2013 Nov 12.

6. LTG Patricia Horoho (US Army Surgeon General). Testimony on Defense Health Program before the Subcommittee on Defense of the House Committee on Appropriations, House of Representatives, 113th Congress, April 2, 2014 (Recorded Version). Retrieved from http://armymedicine.mil/Documents/LTG-Patricia-Horoho-Written-Testimony.pdf. Accessed 27 August 2014.

Key Research Accomplishments

BATTLE Trial

- Poster presented at the AHA EPI/NPAM 2014 Scientific Session and published in *Circulation*:
  - Walizer EM, Vernalis MN, Modlin RE. Influence of CIMT as a motivator for health behavior change in a heart health program. *Circ.* 2014;129:AP126. (, San Francisco, CA, March 19, 2014)

Comprehensive Cardiovascular Risk Assessment and Prevention Program (CHP)

- Paper published in *Sleep Breath*
  - Eliasson A, Kashani M, Modlin R, Howard R, Vernalis M. Fatigued on Venus, Sleepy on Marsð Gender and racial differences in symptoms of sleep apnea. *Sleep Breath.* 2014 Mar 15. [Epub ahead of print]

- Abstract accepted for presentation at CHEST 2014
  - Eliasson AH, Kashani MD, Doody MM, Jones MK, Vernalis MN. Fatigue in Women is a Key Symptom in Evaluation of Sleep Apnea. *CHEST*, Oct 2014; Austin, TX.

- Abstract submitted for presentation at AHA Scientific Session 2014
  - Kashani M, Eliasson A, Engler R, Fuller C, Vernalis M. Prehypertension coexists with CVD risk factors. *AHA Scientific Session 2014*. November 2014.

- Abstracts submitted for presentation at American College of Cardiology, 64th Annual Scientific Session 2015
  - Engler, RJ, Vernalis MN, Mamula KA, Blackburn HL, Kashani M, Ellsworth DL. Lipoprotein Insulin Resistance Index (LP-IR) Changes with Weight Loss Following 1 Year Low Fat Vegan Diet. *American College of Cardiology, 64th Annual Scientific Session*, March 14-16, 2015, San Diego, CA.
  - Kashani M, Eliasson A, Engler R, Turner E, Tschiltz N, Grunewald M, Halsey J, Fuller C, Villines T, Vernalis M. Prediabetes Reversal Using a Novel Comprehensive Health Model. *American College of Cardiology, 64th Annual Scientific Session*, March 14-16, 2015, San Diego, CA.

- Abstract submitted for presentation at AHA Epi/Lifestyle Scientific Session 2015
  - Kashani M, Eliasson A, Engler R, Fuller C, Villines T, Vernalis M. Modest Elevation in Blood Pressure is a Red Flag for Cardiovascular Disease Risk. *AHA Epidem-Lifestyle 2015*, March 2015.

- Development and implementation of Executive Medicine Program at request of OTSG.

Validation of the ICHP Cardiovascular Risk Score

- Paper published in *J of Cardiovasc Nurs*
  - Kashani M, Eliasson A, Bailey K, Vernalis M. A systematic approach incorporating family history improves identification of cardiovascular disease risk. *J of Cardiovasc Nurs*. 2014 May 20. [Epub ahead of print]

ZENITH: Recruitment initiated

Global/CRC Completion

- Abstract submitted to the American Cardiology of Cardiology:
  - Ellsworth DL, Mamula KA, Blackburn HL, Engler RJM, Vernalis MN. Cardiac lifestyle interventions differing in dietary stringency improve insulin resistance through changes in lipoprotein profiles. American College of Cardiology, 64th Annual Scientific Session, March 14-16, 2015, San Diego, CA.

- Paper published in *Circulation: Cardiovascular Genetics*:
  - Ellsworth DL, Croft DT Jr, Weyandt J, Sturtz LA, Blackburn HL, Burke A, Haberkorn MJ, McDyer FA, Jellema GL, van Laar R, Mamula KA, Vernalis MN. Intensive cardiovascular risk reduction induces sustainable changes in expression of genes and pathways important to vascular function. Circ Cardiovasc Genet 2014;7:151-160.

- Paper prepared and will be submitted to *Obesity*:
  - Ellsworth DL, Mamula KA, Blackburn HL, McDyer FA, Jellema GL, van Laar R, Engler RJ, Vernalis MN. Importance of substantial weight loss for altering gene expression during intensive cardiovascular lifestyle modification. Obesity.

- Paper prepared and will be submitted for publication:
  - Mamula KA, Vernalis MN, Ellsworth DL. Attrition from lifestyle modification programs for cardiovascular risk reduction: gender specific considerations and predictors.

Metabolic and Molecular Biology Studies in Surgical Interventions for Morbid Obesity

- Poster presented at the Obesity Society meetings:
  - Blackburn HL, Mamula KA, Haberkorn MJ, Burke A, Slavik JE, Sann NJ, Marley KR, Vernalis MN, Ellsworth DL. Differential effectiveness of laparoscopically-adjustable gastric banding versus lifestyle modification for modifying plasma lipoprotein profiles. Obesity 2013: 31st Annual Scientific Meeting, November 11-16, 2013, Atlanta, GA.

Global Profiling Long-term Study: Completed gene expression assays

Windber Research Institute transitioned to new Institutional Review Board

- All protocols for Task 3.2, Task 4, Task 6, Task 7, and Task 8 were submitted to and approved by the Chesapeake IRB.

Reportable Outcomes

Published Manuscripts (See Appendix A)/Abstracts:

Ellsworth DL, Croft DT Jr, Weyandt J, Sturtz LA, Blackburn HL, Burke A, Haberkorn MJ, McDyer FA, Jellema GL, van Laar R, Mamula KA, Vernalis MN. Intensive cardiovascular risk reduction induces sustainable changes in expression of genes and pathways important to vascular function. *Circ Cardiovasc Genet.* 2014;7:151-160. Epub 2014 Feb 21.

Eliasson A, Kashani M, Modlin R, Howard R, Vernalis M. Fatigued on Venus, Sleepy on Marsð Gender and racial differences in symptoms of sleep apnea. *Sleep Breath*. 2014 Mar 15. [Epub ahead of print]

Kashani M, Eliasson A, Bailey K, Vernalis M. A Systematic Approach incorporating Family History Improves Identification of Cardiovascular Disease Risk. *J of Cardiovasc Nurs.* 2014 May 20. (Epub ahead of print)

Walizer EM, Vernalis MN, Modlin RE. Influence of CIMT as a motivator for health behavior change in a heart health program. *Circ.* 2014;129:AP126.

Manuscripts-to be submitted:

Decewicz A, Hicks M, Mamula KA, Burke A, Haberkorn MJ, Patney HL, Vernalis MN, Ellsworth DL. SNPs associated with plasma triglyceride levels influence response during intensive cardiovascular risk reduction.

Ellsworth DL, Mamula KA, Blackburn HL, McDyer FA, Jellema GL, van Laar R, Engler RJ, Vernalis MN. Importance of substantial weight loss for altering gene expression during intensive cardiovascular lifestyle modification. Obesity (to be submitted).

Kashani M, Eliasson A, Modlin R, Vernalis M. Cardiovascular Health Program Increases Self-Efficacy.

Mamula KA, Vernalis MN, Ellsworth DL. Attrition from Lifestyle Modification Programs for Cardiovascular Risk Reduction: Gender Specific Considerations and Predictors.

Miller EJ, Mamula KA, Leng L, Piecychna M, Vernalis MN, Bucala R, Ellsworth DL. Cardiovascular disease risk factor modification decreases hs-CRP and macrophage migration inhibitory factor (MIF): Influence of gender.

Saum NS, Halsey JF, Walizer EM, Vernalis MN. Exploring the role and impact of limited mindfulness training in changing diet and exercise behaviors.

Walizer EM, Vernalis MN. Does visual knowledge of increased risk for cardiovascular disease affect lifestyle change program adherence?

Conclusions

Unhealthy lifestyle behaviors are linked to the development of CHD, as well as other chronic diseases. Projections based on combined CVD risk factor impact suggest that favorable lifestyle habits could nearly eliminate the development of CHD and substantially decrease CHD morbidity and mortality. We have demonstrated that comprehensive lifestyle interventions are remarkably efficacious in reducing CVD risk factors and, in many cases, are comparable to pharmacological interventions. We also have shown that molecular change occurs during lifestyle modification, but this change may be transient and may be dependent on maintaining a healthy lifestyle. Future research endeavors from this project will provide new information regarding strategies to improve adoption of healthy lifestyle behaviors, the impact of lifestyle interventions on CVD risk, and the biologic mechanisms through which lifestyle changes exert their influence. Through this research, the DOD has a unique opportunity to identify and address adverse lifestyle behaviors and CVD risk factors early and make cardiovascular health a part of the military culture. A commitment to CV health could prevent cardiac events, reduce the need for costly procedures and hospitalization, improve quality of life and protect the investment of highly trained military personnel.

Appendix A
Published Manuscripts

Intensive Cardiovascular Risk Reduction Induces Sustainable Changes in Expression of Genes and Pathways Important to Vascular Function

Darrell L. Ellsworth, PhD; Daniel T. Croft, Jr., MS; Jamie Weyandt, BS; Lori A. Sturtz, PhD; Heather L. Blackburn, BS; Amy Burke, RN, BSN; Mary Jane Haberkorn, RN; Fionnuala A. McDyer, MS; Gera L. Jellema, MS; Ryan van Laar, PhD; Kimberly A. Mamula, MS; Yaqin Chen, MA; Marina N. Vernalis, DO, FACC

Background—Healthy lifestyle changes are thought to mediate cardiovascular disease risk through pathways affecting endothelial function and progression of atherosclerosis; however, the extent, persistence, and clinical significance of molecular change during lifestyle modification are not well known. We examined the effect of a rigorous cardiovascular disease risk reduction program on peripheral blood gene expression profiles in 63 participants and 63 matched controls to characterize molecular responses and identify regulatory pathways important to cardiovascular health.

Methods and Results—Dramatic changes in dietary fat intake (−61%; $P<0.001$ versus controls) and physical fitness (+34%; $P<0.001$) led to significant improvements in cardiovascular disease risk factors. Analysis of variance with false discovery rate correction for multiple testing ($P<0.05$) identified 26 genes after 12 weeks and 143 genes after 52 weeks that were differentially expressed from baseline in participants. Controls showed little change in cardiovascular disease risk factors or gene expression. Quantitative reverse transcription polymerase chain reaction validated differential expression for selected transcripts. Lifestyle modification effectively reduced expression of proinflammatory genes associated with neutrophil activation and molecular pathways important to vascular function, including cytokine production, carbohydrate metabolism, and steroid hormones. Prescription medications did not significantly affect changes in gene expression.

Conclusions—Successful and sustained modulation of gene expression through lifestyle changes may have beneficial effects on the vascular system not apparent from traditional risk factors. Healthy lifestyles may restore homeostasis to the leukocyte transcriptome by downregulating lactoferrin and other genes important in the pathogenesis of atherosclerosis.

Clinical Trial Registration—URL: www.clinicaltrials.gov. Unique identifier: NCT01805492

(*Circ Cardiovasc Genet*. 2014;7:151-160.)

Key Words: cardiovascular diseases ■ gene expression ■ lifestyle ■ obesity

Cardiovascular disease (CVD) remains the leading cause of death and healthcare burden in the United States, accounting for 1 of every 3 deaths and ≈$313 billion in healthcare-related costs in 2009.[1] Many patients with coronary artery disease (CAD) require expensive surgical interventions, such as coronary artery bypass grafting or percutaneous catheter placement, with significant morbidity and mortality.[2]

Clinical Perspective on p 160

Abundant research has established the relationship between dietary habits and CVD risk,[3,4] and physical activity has been associated with significant reductions in cardiac mortality.[5] Lifestyle modification programs focusing on nutrition and exercise have shown substantial health benefits,[6] in part, by improving endothelial function, reducing cardiovascular events, and slowing or reversing progression of coronary atherosclerosis.[7] Although lifestyle programs are effective in mediating CVD risk through traditional risk factors, little is known about molecular change during intensive lifestyle modification or the significance of molecular responses in long-term CVD risk reduction.

We report the effect of an intensive lifestyle program on peripheral blood gene expression to improve our understanding of cellular and molecular changes that occur during risk reduction in patients with, or at risk for, heart disease. Previous studies have shown that patterns of gene expression in peripheral blood are associated with various CVD phenotypes, including presence of CAD[8] and extent of coronary artery atherosclerosis.[9,10] Our study reveals that gene expression signatures are significantly modulated by rigorous lifestyle behaviors and track with CVD risk profiles over time. These observations suggest that successful and sustained

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From the Integrative Cardiac Health Program (D.L.E., D.T.C., J.W., L.A.S., H.L.B., K.A.M.) and Clinical Breast Care Project (Y.C.), Windber Research Institute, Windber Medical Center, Windber, PA (A.B., M.J.H.); Almac Diagnostics, Craigavon, UK (F.A.M., G.L.J.); ChipDX, New York, NY (R.v.L.); and Integrative Cardiac Health Program, Walter Reed National Military Medical Center, Bethesda, MD (M.N.V.).

The Data Supplement is available at http://circgenetics.ahajournals.org/lookup/suppl/doi:10.1161/CIRCGENETICS.113.000121/-/DC1.

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modulation of gene expression through lifestyle changes may have beneficial effects on vascular health that cannot be discerned from traditional risk factor profiles.

Methods

Participants

The research protocol was approved by the Institutional Review Board at Windber Medical Center. All subjects volunteered to participate and gave written informed consent. Men and women willing to make comprehensive lifestyle changes completed a prospective, nonrandomized clinical intervention to stabilize or reverse progression of heart disease through changes in lifestyle. Entry criteria were (1) diagnosis of CAD, which included stable angina, angioplasty, evidence of ≥50% luminal narrowing on coronary angiogram, acute myocardial infarction, bypass surgery, or stent placement; or (2) ≥2 CAD risk factors: hypertension (systolic pressure >140 mm Hg or diastolic pressure >90 mm Hg), high total cholesterol (>200 mg/dL), diabetes mellitus, obesity defined as body mass index (BMI) ≥30, or family history of heart disease in parents or siblings. Controls receiving only standard care from their primary physicians were prospectively matched to program participants based on age, sex, and disease status.[11]

Traditional CAD Risk Factors and Diet

Participants were enrolled on an ongoing basis in a lifestyle intervention that consisted of 4 components: (1) low-fat vegetarian diet (<10% of calories from fat), (2) 180 minutes/wk of moderate aerobic exercise, (3) 1 hour of stress management each day, and (4) weekly group support sessions. Demographic and clinical information was obtained by standard questionnaires at baseline, 12 weeks, and 52 weeks. Physiological and biochemical variables were assessed as previously described.[12,13] Dietary data were collected from self-reported 72-hour dietary recall questionnaires. Food Processor v8.4.0 (ESHA Research) was used to determine daily caloric intake and nutrient composition.

Blood Collection, RNA Preparation, and Microarray Analysis

Peripheral blood was obtained from participants at each time point using the PAXgene Blood RNA System (Qiagen). RNA was isolated and quantified following the Qiagen protocol. Globin mRNA transcripts were depleted from a portion of each total RNA sample using the GLOBINclear-Human kit (Ambion). Globin-depleted RNA aliquots (1 μg) were amplified using the MessageAmp II aRNA Amplification System (Ambion). Resulting double-stranded cDNA was purified, amplified, and labeled with biotin-11-uridine-5′-triphosphate. Labeled aRNA (15 μg) was then fragmented and hybridized to GeneChip Human Genome U133A 2.0 arrays (Affymetrix) and scanned on a GeneChip Scanner 3000. Samples were run in batches for globin reduction (n=12), RNA amplification (n=12), and microarray analysis (n=6), keeping all 3 time points for each participant together in the same batch to minimize technical artifact. All gene expression data have been deposited in the Gene Expression Omnibus, series accession number GSE46097 (http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE46097).

Informatics and Analysis

Statistical analysis of CVD risk factors was conducted using JMP (v9.0). Baseline levels for intervention and matched controls were compared using a matched pairs *t* test, and change in risk factors over time was assessed with a Wilcoxon signed-rank test, which analyzed differences in risk factor response among the matched pairs.

Partek Genomics Suite v6.5 (Partek Incorporated) was used to analyze gene expression data from the 378 CEL files, which all passed standard quality control assessment. Duplicate blood samples collected at each time point from 7 random participants indicated high repeatability of the microarray data (average Pearson correlation of normalized intensities was 0.992±0.006; range, 0.969–0.996). Paired *t* tests identified 9 genes that were excluded from further analysis because of significant differences in expression among duplicate samples (Table I in the Data Supplement).

Using 1-way analysis of variance with false discovery rate correction for multiple testing, we first compared baseline levels of gene expression between lifestyle participants and controls and then examined expression changes from baseline to week 12 and baseline to week 52 in lifestyle participants, and separately in controls, to determine genes that changed significantly over time in each group. Stringent gene lists were generated through combined significance (FDR $P<0.05$) and expression change (≥1.1-fold) filtering. Pairwise Pearson product-moment correlations between changes in gene expression and changes in CVD risk factors were calculated using JMP. Functional enrichment analysis was performed on stringent gene lists to identify biological processes controlled by differentially expressed genes. Gene set enrichment analysis, using BRB-ArrayTools v4.2.1 on the Kyoto Encyclopedia of Genes and Genomes database, identified differential expression between groups of genes with common biological function or regulation.[14] To distinguish the effects of the program from the potential influence of medications on gene expression, ancillary analyses were conducted that included only participants who were not taking or did not change the brand or dosage of medications in the following categories: angiotensin-converting enzyme inhibitors, β-blockers, calcium channel blockers, or lipid-lowering drugs.

Transcript Validation by Quantitative Reverse Transcription Polymerase Chain Reaction

Total RNA (500 ng) was reverse transcribed using the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems) and subjected to quantitative reverse transcription polymerase chain reaction using TaqMan Gene Expression Assays (Applied Biosystems). All target gene expression levels were normalized to the housekeeping gene GAPDH. Samples were run in duplicate for each assay, and the mean value was analyzed by the $\Delta\Delta C_T$ method.[15,16] A repeated measures analysis of variance then determined whether fold-change in expression between time points for each gene was statistically significant. Additional materials and methods are described in the Data Supplement.

Results

The average age of lifestyle participants was 60.3 years (range, 44.5–78.4 years). Many participants entered the program with clinically relevant disorders: 41% had hypertension, 60% were clinically obese, and 54% had high cholesterol. At baseline, participants had higher average BMI (32.6±6.7 versus 28.4±3.9) and triglycerides (187±101 versus 133±73 mg/dL) but lower exercise capacity (24.9±7.4 versus 36.7±11.9 mL per kg per minute) than controls ($P<0.01$), despite the prospective matching strategy (Table II in the Data Supplement). Participants who completed the program tended to be older (60.3±9.3 versus 55.3±11.3 years of age) and have higher systolic blood pressure (137±17 versus 131±19 mm Hg) than those who dropped out ($P<0.05$; Table III in the Data Supplement).

Traditional CAD Risk Factors and Diet

The program resulted in substantial reductions in the number of hypertensive (41% down to 17%), obese (60%–37%), and dyslipidemic (54%–37%) patients. In the first 12 weeks, participants showed dramatic improvement in most dietary and CVD risk factors, but little change occurred in controls (Table 1). At 52 weeks, participants maintained significantly lower daily fat intake (−60%; $P<0.001$ compared with matched controls) and higher carbohydrate consumption (+30%; $P<0.001$ versus matched controls). Improvements in BMI (−9%; $P<0.001$), triglycerides (−7%; $P<0.01$), and physical fitness (+38%; $P<0.001$) remained significant compared with matched nonintervention

Table 1. Change in Dietary and Cardiovascular Risk Factors in Participants and Controls

	Controls (n=63)				Participants (n=63)				
Measure	Baseline	12 Weeks	52 Weeks	% Change B-W52*	Baseline	12 Weeks	52 Weeks	% Change B-W52*	Matched Pairs P†
Dietary									
Calories	1750±547	1719±591	1633±462	−6.7	1985±763	1505±293‡	1700±442§	−14.4	0.369
% Carbohydrate‖	49.3±10.0	49.3±7.3	49.9±10.1	+1.2	54.5±10.8	71.2±3.8‡	71.1±3.6‡	+30.4	<0.001
% Fat	32.4±9.3	32.6±6.3	31.7±8.2	−2.2	29.1±10.3	11.2±1.9‡	11.4±3.0‡	−60.7	<0.001
Physiological									
BMI, kg/m²‖	28.4±3.9	28.1±4.1	28.6±4.2	+0.8	32.6±6.7	30.2±6.1‡	29.6±6.2‡	−9.4	<0.001
SBP, mm Hg	134±18	128±15§	126±13‡	−5.7	139±16	124±16‡	129±17‡	−7.6	0.277
DBP, mm Hg	79.3±10.3	77.7±8.6	77.4±8.2	−2.4	82.2±9.9	73.5±8.8‡	76.2±9.2‡	−7.3	0.064
LDL, mg/dL	111±36	107±34	110±36	−1.5	116±42	101±33‡	114±35	−1.3	0.958
TCH, mg/dL	192±46	189±45	190±46	−1.1	200±49	173±42‡	192±43§	−3.9	0.207
TG, mg/dL‖	133±73	151±146	145±77	+8.6	187±101	168±82	174±102	−7.0	0.005
EC (Vo_2 max)‖	36.5±11.8	37.5±11.2	36.4±11.1	−0.1	25.0±8.0	32.0±8.3‡	34.6±10.0‡	+38.4	<0.001

Data presented as mean±SD. There were 36 women and 27 men in each group; 3.7% missing data. BMI indicates body mass index; DBP, diastolic blood pressure; EC, exercise capacity; LDL, low-density lipoprotein; SBP, systolic blood pressure; TCH, total cholesterol; and TG, triglycerides.

*Percent change from baseline to 52 wk.

†From a Wilcoxon signed-rank test for matched pairs comparing changes from baseline to 52 wk in participants and matched controls.

‡$P<0.001$ compared with baseline by a paired t test.

§$P<0.05$ compared with baseline by a paired t test.

‖Baseline values significantly different ($P<0.05$) between participants and controls based on a matched pairs t test.

controls, but systolic blood pressure and lipids showed regression toward pretreatment levels.

Gene Expression

Levels of gene expression were similar between participants and controls at baseline—only 1 (214731_at) of 22 277 probes showed a significant difference (FDR $P<0.05$) between groups. Stringent differential analysis identified 26 unique genes (3 upregulated and 23 downregulated) that changed significantly in expression after 3 months of intervention (Table IV in the Data Supplement). By 1 year, 143 characterized genes were significantly upregulated (n=44) or downregulated (n=99) from baseline in lifestyle participants (Table 2; Table V in the Data Supplement). Downregulation of gene expression during lifestyle change occurred far more frequently than expected by chance. Using a binomial distribution calculated as a probability mass function with $P=0.5$, the probability was 3.9×10^{-5} for observing 23 of 26 genes downregulated at 12 weeks and 1.4×10^{-6} for 99 of 143 genes downregulated at 52 weeks. Validation using quantitative reverse transcription polymerase chain reaction confirmed the overall accuracy of the array-based expression results for the transcripts tested (Table 3). In contrast to lifestyle participants, control subjects showed no change in gene expression after 12 weeks (0 genes) and little change by 52 weeks (21 genes; Table VI in the Data Supplement).

Correlations Between CVD Risk Factors and Gene Expression

Throughout the program, many genes exhibiting the largest fold-changes in expression were significantly correlated with BMI (Figure 1). Notably, few genes correlated with blood pressure or plasma lipids after 12 weeks. Dysregulation of several genes was associated with improvement in triglycerides (−10%) during the first 3 months but was not associated after the 12-week examination when triglyceride levels regressed toward baseline.

Functional Analysis

Functional enrichment analysis indicated that genes showing significant changes in expression during the intervention function mainly in immune response and cholesterol storage (Table VII in the Data Supplement). Genes with the greatest changes in expression at 12 weeks showed regression by 52 weeks (Figure 2). Expression of the majority of immune response genes (65%) closely paralleled the substantial improvement followed by regression pattern observed for some traditional risk factors. In contrast, many cholesterol/lipid homeostasis genes (67%) showed a pattern of continual change throughout the program similar to BMI.

Gene set enrichment analysis provided additional insight into molecular pathways regulated by cardiovascular risk factor modification but that were subtle at the individual gene level. Table 4 shows Kyoto Encyclopedia of Genes and Genomes pathways with Efron–Tibshirani max mean statistic[17] ≤0.001 at 12 and 52 weeks. Pathways affected early in lifestyle modification were related to carbohydrate metabolism, glycoprotein hormone levels, and cytokine production, whereas pathways altered later control steroid hormones, cell mobility, and signal transduction and inflammation.

Effects of Medications

Participants were taking 79 different prescription medications at baseline. To determine whether common cardiovascular medications affected gene expression, we examined subgroups of participants based on medication use. In these analyses, changes in expression in participants not taking cardiovascular medications or whose medication levels did not change during

Table 2. Genes Showing Greatest Fold-Change in Expression During CVD Risk Factor Modification

Probe ID	Gene Name	Symbol	Fold-Change	Gene Ontology Biological Process*
202018_s_at†	Lactotransferrin	LTF	–1.67	Immune response, ion transport, iron homeostasis
221748_s_at	Tensin 1‡	TNS1	–1.55	Cell migration, cell-substrate junction assembly
212531_at†	Lipocalin-2	LCN2	–1.47	Transporter activity; binding§
206676_at†	Carcinoembryonic antigen–related CAM8	CEACAM8	–1.44	Immune response
214407_x_at	Glycophorin B (MNS blood group)‡	GYPB	–1.41	Signal transduction; receptor activity§
206698_at	X-linked Kx blood group	XK	–1.41	Amino acid transport
206665_s_at	BCL2-like 1	BCL2L1	–1.39	Response to hypoxia/oxidative stress, apoptosis
203502_at	2,3-bisphosphoglycerate mutase	BPGM	–1.37	Carbohydrate metabolism, glycolysis, respiration
203115_at	Ferrochelatase‡	FECH	–1.35	Cholesterol metabolism, metabolites/energy
207802_at†	Cysteine-rich secretory protein 3	CRISP3	–1.32	Immune response, defense response
208470_s_at†	Haptoglobin/haptoglobin-related protein‡	HP/HPR	–1.30	Defense response, proteolysis, iron homeostasis
212768_s_at†	Olfactomedin 4	OLFM4	–1.29	Cell adhesion, protein binding
213446_s_at	IQ motif containing GTPase-activating protein 1‡	IQGAP1	–1.28	Small GTPase-mediated signal transduction
208632_at	Ring finger protein 10	RNF10	–1.28	Transcription, Schwann cell proliferation
221627_at	Tripartite motif containing 10	TRIM10	–1.28	Erythrocyte differentiation; protein/ion binding§
218418_s_at	KN motif and ankyrin repeat domains 2	KANK2	–1.28	Transcription apoptosis, cell proliferation
217878_s_at	Cell division cycle 27 homolog‡	CDC27	–1.27	Cell proliferation, cell division
210244_at†	Cathelicidin antimicrobial peptide	CAMP	–1.27	Defense response
200615_s_at	Adaptor-related protein complex 2, β1	AP2B1	–1.26	Protein transport, defense response
205557_at†	Bactericidal/permeability-increasing protein	BPI	–1.25	Immune response; lipid binding§
211993_at	WNK lysine–deficient protein kinase 1	WNK1	–1.25	BP regulation, phosphorylation, ion transport

Stringent gene list of changes at 52 wk with combined significance (FDR $P<0.05$) and expression change (≥1.25-fold) filtering. BCL2 indicates B-cell CLL/lymphoma 2; BP, blood pressure; CAM, cell adhesion molecule; and CVD, cardiovascular disease.

*Derived from NetAffx Analysis Center (http://www.affymetrix.com/analysis/index.affx).

†Probes were significant at 12 wk.

‡Three probes for TNS1 and GYPB and 2 probes for FECH, HP/HPR, IQGAP, and CDC27 showed a significant fold-change from baseline to 52 wk.

§Gene Ontology molecular function.

the study were similar to changes in all participants, showing that prescription medication use did not have significant effects on gene expression during lifestyle change (Table 5).

Discussion

Participants who completed a comprehensive lifestyle intervention designed to reverse or stabilize progression of CAD dramatically changed their dietary habits and significantly increased physical activity, which led to substantial weight loss during 1 year. We have previously shown that CVD risk reduction through intensive lifestyle change has positive effects on vascular and mental health by reducing cardiometabolic risk,[12] modulating plasma lipoprotein profiles,[13] and improving clinical measures of depression and stress.[18] Here, we show that

Table 3. Validation of Differential Gene Expression During CVD Risk Factor Modification

	Controls (n=45)				Participants (n=44)				
Gene	12-Week Fold-Change	*P* Value*	52-Week Fold-Change	*P* Value†	12-Week Fold-Change	*P* Value*	52-Week Fold-Change	*P* Value†	Time×CS-CN *P* Value‡
LTF	+0.67	0.469	+0.89	0.328	–2.01	0.002	–1.72	0.026	0.037
LCN2	+0.58	0.202	+0.62	0.187	–0.91	0.078	–0.98	0.057	0.020
CEACAM8	+1.11	0.274	+1.22	0.279	–3.44	0.006	–1.75	0.049	0.010
CRISP3	+1.02	0.230	+1.28	0.242	–2.35	0.005	–1.56	0.004	0.007
HP	+0.15	0.484	–0.07	0.983	–0.96	0.007	–1.18	<0.001	0.008
OLFM4	+1.87	0.102	–0.30	0.625	–6.97	0.011	–4.56	0.071	0.026
CAMP	+0.44	0.230	+0.07	0.562	–1.10	0.007	–1.32	0.033	0.012
BPI	+0.16	0.519	–0.17	0.914	–1.18	0.017	–0.81	0.044	0.040

Validation using quantitative reverse transcription polymerase chain reaction and the $\Delta\Delta C_T$ method was conducted on 45 controls and 44 participants with sufficient RNA remaining for analysis. CVD indicates cardiovascular disease; CN, controls; and CS, cases (or participants).

**P* value comparing 12 wk to baseline using a paired *t* test.

†*P* value comparing 52 wk to baseline using a paired *t* test.

‡Between-group *P* value for time variable using a repeated measures analysis of variance comparing program participants (CS) with controls (CN).

B-W12	BMI −7.5%	SBP −11.1%	DBP −10.7%	LDL −12.9%	TCH −13.4%	TG −10.1%	EC +28.1%
LTF	+0.44	+0.12	+0.18	+0.23	+0.23	+0.11	−0.09
TNS1	+0.11	+0.01	+0.05	−0.06	−0.13	−0.17	+0.09
LCN2	+0.32	+0.12	+0.08	+0.09	+0.09	+0.08	−0.01
CEACAM8	+0.47	+0.06	+0.03	+0.32	+0.26	+0.09	−0.03
GYPB	+0.04	−0.04	−0.05	−0.05	−0.10	−0.11	+0.13
XK	+0.04	−0.02	−0.02	−0.03	−0.09	−0.18	+0.17
BCL2L1	−0.04	+0.03	+0.07	−0.06	−0.15	−0.27	+0.10
BPGM	+0.10	−0.06	+0.01	−0.01	−0.05	−0.11	+0.18
FECH	+0.09	−0.04	+0.01	−0.12	−0.15	−0.12	+0.12
CRISP3	+0.41	+0.02	+0.06	+0.21	+0.28	+0.27	−0.14
HP/HPR	+0.32	+0.07	+0.24	+0.22	+0.24	+0.01	−0.01
OLFM4	+0.43	−0.01	+0.05	+0.29	+0.29	+0.20	+0.06
IQGAP1	+0.03	+0.02	+0.20	+0.04	−0.02	−0.27	−0.11
RNF10	+0.07	−0.03	+0.04	−0.09	−0.19	−0.26	+0.05
TRIM10	+0.05	−0.06	−0.05	−0.06	−0.16	−0.25	+0.10
KANK2	−0.01	−0.07	−0.01	−0.12	−0.20	−0.19	+0.09
CDC27	+0.05	−0.10	+0.01	−0.10	−0.15	−0.26	−0.07
CAMP	+0.29	0.00	+0.04	+0.13	+0.18	+0.17	−0.01
AP2B1	+0.07	−0.09	+0.15	+0.02	−0.09	−0.27	+0.07
BPI	+0.34	+0.21	+0.17	+0.10	+0.07	−0.02	+0.03
WNK1	+0.01	−0.03	+0.03	−0.12	−0.19	−0.25	+0.09

W12-52	BMI −2.0%	SBP +4.0%	DBP +3.8%	LDL +13.3%	TCH +11.1%	TG +3.4%	EC +8.0%
LTF	+0.24	+0.12	+0.06	−0.19	−0.11	+0.23	−0.26
TNS1	+0.39	+0.11	+0.03	−0.14	−0.09	+0.11	−0.32
LCN2	+0.29	+0.12	+0.05	−0.23	−0.19	+0.07	−0.34
CEACAM8	+0.27	+0.06	+0.01	−0.18	−0.14	+0.15	−0.26
GYPB	+0.50	+0.07	+0.03	−0.24	−0.22	0.00	−0.39
XK	+0.46	+0.06	−0.01	−0.22	−0.22	−0.02	−0.36
BCL2L1	+0.33	+0.05	−0.03	−0.07	−0.06	+0.02	−0.31
BPGM	+0.49	+0.03	−0.05	−0.20	−0.18	−0.01	−0.35
FECH	+0.48	+0.09	−0.01	−0.18	−0.18	−0.01	−0.37
CRISP3	+0.26	−0.08	−0.05	−0.22	−0.20	+0.08	−0.18
HP/HPR	+0.32	−0.05	−0.03	−0.32	−0.27	+0.09	−0.18
OLFM4	+0.16	−0.01	0.00	−0.15	−0.13	+0.14	−0.17
IQGAP1	0.00	+0.26	+0.18	+0.10	+0.11	+0.10	−0.03
RNF10	+0.29	+0.12	+0.04	−0.16	−0.12	+0.09	−0.33
TRIM10	+0.36	+0.07	−0.02	−0.21	−0.20	+0.02	−0.38
KANK2	+0.37	+0.08	−0.09	−0.12	−0.12	−0.02	−0.35
CDC27	+0.13	+0.12	+0.04	−0.03	−0.01	+0.05	−0.32
CAMP	+0.34	+0.02	+0.03	−0.31	−0.29	−0.02	−0.29
AP2B1	+0.26	+0.08	−0.05	−0.08	−0.04	+0.15	−0.29
BPI	+0.25	+0.17	+0.08	−0.29	−0.27	+0.02	−0.24
WNK1	+0.14	+0.16	0.00	+0.03	+0.07	+0.18	−0.17

B-W52	BMI −9.4%	SBP −7.6%	DBP −7.3%	LDL −1.3%	TCH −3.9%	TG −7.0%	EC +38.4%
LTF	+0.46	+0.16	+0.08	+0.18	+0.25	+0.14	−0.35
TNS1	+0.38	+0.13	+0.01	+0.05	+0.08	+0.01	−0.26
LCN2	+0.34	+0.20	+0.05	+0.15	+0.16	+0.08	−0.30
CEACAM8	+0.38	+0.10	+0.07	+0.32	+0.33	+0.06	−0.30
GYPB	+0.39	+0.14	+0.05	0.00	−0.02	−0.04	−0.25
XK	+0.32	+0.12	+0.09	+0.07	+0.05	−0.11	−0.20
BCL2L1	+0.18	+0.10	−0.12	+0.08	+0.06	−0.06	−0.21
BPGM	+0.35	+0.11	+0.10	+0.09	+0.07	−0.13	−0.21
FECH	+0.32	+0.16	+0.01	+0.08	+0.07	−0.09	−0.18
CRISP3	+0.33	−0.02	+0.12	+0.20	+0.24	+0.12	−0.18
HP/HPR	+0.46	+0.01	+0.25	+0.14	+0.04	−0.11	−0.10
OLFM4	+0.35	−0.02	+0.06	+0.15	+0.14	+0.02	−0.18
IQGAP1	+0.20	+0.12	+0.04	+0.02	+0.01	0.00	−0.33
RNF10	+0.38	+0.10	−0.05	+0.02	+0.05	+0.03	−0.29
TRIM10	+0.35	+0.12	0.00	0.00	0.00	−0.10	−0.22
KANK2	+0.32	+0.09	−0.10	+0.02	+0.02	−0.12	−0.21
CDC27	+0.11	−0.02	−0.11	−0.16	−0.16	−0.01	−0.28
CAMP	+0.37	+0.15	+0.21	+0.17	+0.16	+0.04	−0.10
AP2B1	+0.41	+0.07	−0.13	+0.04	+0.05	−0.05	−0.26
BPI	+0.29	+0.23	+0.17	+0.24	+0.19	−0.07	−0.18
WNK1	+0.20	+0.08	−0.06	−0.06	−0.03	−0.02	−0.16

Figure 1. Pairwise correlations for changes in cardiovascular disease risk factors and gene expression from baseline to 12 weeks (**top**), week 12 to week 52 (**middle**), and baseline to week 52 (**bottom**) during intensive lifestyle modification. Percentages in column headings represent degree of change for each risk factor during the corresponding time interval. Coefficients highlighted in dark green were significant at $P<0.001$, light green $P<0.05$. Risk factor percent changes are group averages from Table 1; changes in gene expression were calculated as a percent change for each gene at week 12 and week 52 using raw expression data. Stringent gene list of changes at 52 weeks with combined significance (FDR $P<0.05$) and expression change (≥1.25-fold) filtering. BMI indicates body mass index; DBP, diastolic blood pressure; EC, exercise capacity; LDL, low-density lipoprotein; SBP, systolic blood pressure; TCH, total cholesterol; and TG, triglycerides.

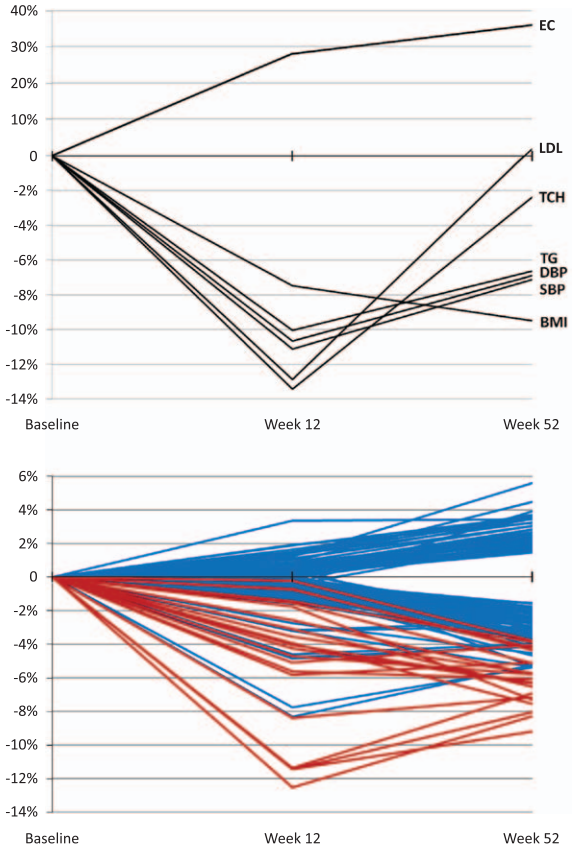

Figure 2. Changes in cardiovascular disease (CVD) risk factors (**top**) and levels of expression for genes differentially regulated during intensive CVD risk reduction (**bottom**). Blue lines, FDR $P<0.05$ and fold-change ≥1.1 but <1.25; red lines, FDR $P<0.05$ and fold-change ≥1.25 at 52 weeks. BMI indicates body mass index; DBP, diastolic blood pressure; EC, exercise capacity; LDL, low-density lipoprotein; SBP, systolic blood pressure; TCH, total cholesterol; and TG, triglycerides.

intensive lifestyle behaviors also modulate gene expression in peripheral blood, suggesting potential CVD risk-reduction mechanisms involving leukocyte function in innate immunity, lipid homeostasis, and inflammation.

Lifestyle modification has been shown to be effective in improving clinically relevant CVD risk factors; however, the extent, persistence, and significance of molecular change accompanying CVD risk reduction are not well known. Daily macronutrients can influence short-term changes in genes related to inflammation, carbohydrate metabolism, and immune function,[19] whereas long-term dietary composition may affect genes and pathways regulating development of atherosclerosis and CVD.[20] Similarly, physical activity induces a variety of rapid biophysical and biochemical responses, including altered expression of genes related to oxidative stress, signal transduction, and inflammation.[21,22] Because expression of diet- and exercise-responsive genes tends to be transient in nature, little is known about the long-term clinical significance of these changes.

During lifestyle modification, participants successfully adopted healthy lifestyle behaviors including a low-fat diet and increased physical activity, which may be important drivers of molecular change. In our analysis of individual

Table 4. KEGG Pathways Differentially Expressed During CVD Risk Factor Modification

ID	Name	No. Genes	Function
Baseline: wk 12			
hsa05120	Epithelial cell signaling in Hpy infection	53	Gene expression and proinflammatory cytokine production in gastric mucosa
hsa04912	GnRH signaling pathway	64	Synthesis/release of gonadotropins; gene expression, stress response
hsa00640	Propanoate metabolism	26	Carboxylic acid metabolism; related to carbohydrate metabolism/glycolysis
Baseline: wk 52			
hsa00150	Androgen and estrogen metabolism	19	Inactivation/catabolism of androgen and estrogen in target tissues
hsa00563	GPI anchor biosynthesis	18	Covalently anchor proteins to cell membranes; signal transduction, inflammation
hsa04810	Regulation of actin cytoskeleton	136	Cellular processes associated with membrane dynamics, cell migration/motility

The Efron–Tibshirani max mean statistic for all pathways was ≤0.001. Available at http://www.genome.jp/kegg/pathway.html. Only galactose metabolism and calcium signaling pathways were differentially expressed in controls at 52 wk. CVD indicates cardiovascular disease; GnRH, gonadotropin-releasing hormone; GPI, glycosylphosphatidylinositol; Hpy, *Helicobacter pylori*; and KEGG, Kyoto Encyclopedia of Genes and Genomes.

genes, immune response and lipid homeostasis were enriched functional categories. The drastic reduction in dietary fat intake during the intervention may influence expression of genes related to lipid storage and transport. Similarly, the predominant downregulation of immune/defense response genes may reflect lower psychological stress and improved vascular health.

Single-gene analysis may miss important effects of lifestyle change on complex molecular pathways; therefore, we conducted gene set enrichment analysis to overview biological processes relevant to CVD risk reduction. Pathways significantly altered were related to physiological changes during the program. The gonadotropin-releasing hormone signaling pathway and the androgen and estrogen metabolism pathway

Table 5. Effects of Medications on Gene Expression From Baseline to 52 Weeks

Probe ID	Symbol	Fold-Change All Participants (n=63)	Fold-Change With Stable or No Lipid Medications (n=51)*	Fold-Change With Stable or No CVD Medications (n=34)†	Among Group *P*‡
202018_s_at	LTF	–1.67	–1.67	–1.70	0.988
221748_s_at	TNS1	–1.55	–1.51	–1.43	0.953
212531_at	LCN2	–1.47	–1.44	–1.48	0.978
206676_at	CEACAM8	–1.44	–1.48	–1.68	0.368
214407_x_at	GYPB	–1.41	–1.34	–1.26	0.768
206698_at	XK	–1.41	–1.43	–1.36	0.933
206665_s_at	BCL2L1	–1.39	–1.35	–1.31	0.946
203502_at	BPGM	–1.37	–1.40	–1.41	0.961
203115_at	FECH	–1.35	–1.31	–1.28	0.933
207802_at	CRISP3	–1.32	–1.32	–1.43	0.637
208470_s_at	HP/HPR	–1.30	–1.31	–1.24	0.856
212768_s_at	OLFM4	–1.29	–1.20	–1.23	0.540
213446_s_at	IQGAP1	–1.28	–1.25	–1.22	0.951
208632_at	RNF10	–1.28	–1.25	–1.18	0.803
221627_at	TRIM10	–1.28	–1.23	–1.21	0.811
218418_s_at	KANK2	–1.28	–1.22	–1.21	0.890
217878_s_at	CDC27	–1.27	–1.26	–1.22	0.961
210244_at	CAMP	–1.27	–1.26	–1.27	0.996
200615_s_at	AP2B1	–1.26	–1.24	–1.22	0.961
205557_at	BPI	–1.25	–1.22	–1.29	0.723
211993_at	WNK1	–1.25	–1.23	–1.17	0.860

CVD indicates cardiovascular disease.

*Includes only participants not taking lipid-lowering medications or whose lipid-lowering medication levels did not change during the study.

†Includes only participants not taking angiotensin-converting enzyme inhibitors, β-blockers, calcium channel blockers, or lipid-lowering medications or whose medication levels for these drugs did not change during the study.

‡Based on a Kruskal–Wallis nonparametric test comparing change in gene expression from baseline to 52 wk among groups.

regulate steroid hormones and activate diverse signaling pathways in nonpituitary tissues that modulate gene expression, cell proliferation, and stress response.[23] Because estrogen and androgen levels are commonly elevated in obesity and weight loss can significantly lower serum estrogen and testosterone levels,[24] weight reduction may lead to changes in pathways affecting sex hormones. Similarly, the propanoate metabolism pathway is related to carbohydrate metabolism and glycolysis; thus, functional changes may reflect increased carbohydrate consumption during the program.

The *Helicobacter pylori* bacterium colonizes the human gastric mucosa and activates multiple signaling pathways.[25] Weight loss through dietary change has been shown to significantly alter the species composition of the intestinal microbiome,[26] thus activation of the *H. pylori* pathway in the first 12 weeks may reflect changes in gut microbiota because of significant dietary changes. Other pathways involving the actin cytoskeleton and glycosylphosphatidylinositol anchor biosynthesis are related to signal transduction, inflammation, and host–pathogen interactions.[27]

Whole blood RNA isolation systems such as PAXgene accurately capture in vivo transcription profiles but cannot distinguish expression signatures unique to specific cell types. To better understand vascular responses to lifestyle modification, we compared genes that were differentially regulated during CVD risk reduction to expression signatures reported for major leukocyte subpopulations. Genes influenced by lifestyle change were expressed in several cell populations, suggesting that different types of circulating cells with unique and specialized functions may be involved in vascular responses to lifestyle modification (Figure 3).

Neutrophils and T-lymphocytes comprise the most abundant leukocyte populations and play essential roles in inflammation and microbial infection. Genes expressed by these specialized cells were downregulated during lifestyle modification, which provides insight into their vascular function and potential role in mediating cardiovascular risk. In particular, neutrophil lactoferrin (LTF; or lactotransferrin) is a multifunctional glycoprotein that serves an important role in host defense and innate immunity. In the circulatory system, LTF

Figure 3. Congruence between cardiovascular disease (CVD) risk-reduction genes and expression signatures reported for major leukocyte subpopulations or CVD-relevant processes. Squares denote whether genes differentially regulated after 52 weeks of intensive lifestyle modification also were expressed (green squares) or not expressed (red squares) in published profiles. *Palmer et al[28]; †Whitney et al[29]; ‡Cobb et al[30]; §Wang et al[31]; ‖Calvano et al.[32]; #Xiao et al.[33]

released by neutrophils regulates production of reactive oxygen species, recruits immune cells to sites of inflammation, and is positively associated with coronary artery stenosis[34] and risk for fatal ischemic heart disease.[35] LTF gene expression is induced in atherosclerotic plaques of human aortas compared with nonatherosclerotic internal thoracic arteries,[36] and salivary LTF concentrations are 60% lower in elite athletes versus sedentary controls.[37] Importantly, in vitro studies have shown that LTF directly affects leukocyte functions that contribute to CVD, including attenuating leukocyte adhesion to vascular endothelial cells, modulating proinflammatory cytokine expression in endothelial cells, and inhibiting processes essential for vascular dysfunction such as proliferation, migration, and angiogenesis.[38] Such parallel evidence implicating LTF in vascular health increases confidence in the validity of our findings and suggests LTF may be therapeutic in patients with CVD who lead unhealthy lifestyles.

Lipocalin-2 (or neutrophil gelatinase-associated lipocalin) is a proinflammatory glycoprotein released by activated neutrophils in response to inflammatory stimuli.[39] Clinical and experimental studies suggest serum lipocalin-2 levels are elevated in obesity and related metabolic complications[40] and positively associated with CAD and cardiac dysfunction.[41,42] Lipocalin-2 is highly expressed in vascular smooth muscle cells and may function in atherosclerotic plaque development by promoting endothelial activation and vascular leukocyte infiltration.[43] Carcinoembryonic antigen–related cell adhesion molecules are immunoglobulin-related glycoproteins that are glycosylphosphatidylinositol-anchored to the surface of granulocytes (neutrophils and eosinophils), where they regulate activation and release of proinflammatory mediators during inflammation and host immunity.[44] Carcinoembryonic antigen–related cell adhesion molecules have been shown to influence neutrophil adhesion to human umbilical vein endothelial cells.[45]

Changes in blood leukocyte gene expression when immune cell function is accentuated, such as systemic inflammation and severe trauma, provide further insight into regulation of leukocyte function during CVD risk reduction. In response to severe bodily injury and infection, leukocytes significantly upregulate expression of numerous genes involved in inflammation and innate immunity.[32,33] Interestingly, genes showing some of the greatest fold increases in expression during severe trauma (LTF, matrix metallopeptidase 8, and haptoglobin) were significantly downregulated during lifestyle change. Lifestyle modification thus may have beneficial effects on vascular health by reducing expression of proinflammatory genes associated with activation of neutrophil granulocytes.

In this study, we controlled for many covariates known to influence blood-based gene expression profiles,[29,46] such as age, sex, time of day, and fasting status, through matching and experimental design. Another complicating factor common among patients with CVD is medication use. Many participants entered the program in poor cardiovascular health, with hypertension, obesity, and hyperlipidemia and, as a result, were taking several prescription medications. These medications may affect cellular function and alter patterns of gene expression in peripheral blood,[47] thus confounding the true effects of lifestyle change. Our analysis indicated that common CVD medications did not have significant effects on peripheral blood gene expression and suggest that alterations in individual genes and multigene pathways were attributable to lifestyle changes.

We showed that intensive lifestyle modification can significantly alter the expression of numerous genes associated with leukocyte function, vascular inflammation, and lipid homeostasis. Fold-changes we observed during a 1-year period in patients undergoing lifestyle modification were comparable in magnitude to differences in expression reported for patients with CVD compared with healthy controls.[8,10] Similar to traditional risk factors, however, these molecular changes seem dynamic, and persistence over time may depend on long-term adherence to healthy behaviors. The number of significantly altered genes increased >5-fold from week 12 to week 52, suggesting that patients who maintain healthy lifestyle behaviors for longer periods of time are likely to experience more diverse molecular change than patients participating in short-term activities. In addition, some conventional risk factors and gene expression profiles showed regression toward baseline after 12 weeks, which corresponded with a lower percentage of participants meeting compliance targets, particularly for exercise and stress management (Table VIII in the Data Supplement). Adherence to cardiovascular treatment regimens involving lifestyle change is particularly difficult, and many patients usually adhere only partially to programmatic goals.[48] Thus, personal motivation and strict adherence are key factors for successful long-term cardiovascular benefit.

Limitations

Intensive lifestyle programs for CVD risk reduction involve demanding behavioral changes that require motivation and a significant time commitment, which likely restrict the applicability of such programs to patients in general. Accordingly, it was impractical to use a randomized study design, which may limit the conclusions that can be drawn from the data, although well-designed case–control studies may be similar to randomized trials for estimating treatment effects.[49] We analyzed the data using a per-protocol (on-treatment) approach but included all patients who completed the program regardless of whether they strictly adhered to the program guidelines. The multifaceted nature of the program precluded us from precisely defining the relative contribution of each component in driving molecular and physiological changes; however, the correlation analysis indicated that many observed changes in gene expression may be attributable to weight loss and physical activity. Furthermore, we could not evaluate long-term changes in gene expression and CVD risk factors beyond 1 year, and we could not assess whether the observed results are achievable outside a controlled clinical environment.

During the intervention, our patients remained under the care of their primary physicians, who may have prescribed medications other than cardiovascular medications. We conducted a subgroup analysis to account for potential effects of common cardiovascular medications on patterns of gene expression, but it is possible that other medications not examined in these analyses influence leukocyte gene transcription.

Peripheral blood is a complex tissue with diverse cell populations whose relative abundance is dynamic over time. Gene

expression studies using whole blood cannot distinguish the effects of cellular demographics from signatures of physiological response. To address this issue, we examined published expression signatures of major leukocyte populations to infer specific cell types involved in response to lifestyle modification; however, rare cell types not examined may play an important role in CVD risk reduction.

Conclusions

CVD prevention through intensive lifestyle changes leads to improvements in clinically relevant cardiac risk factors that may be important in the pathogenesis of atherosclerosis.[50] However, the extent and significance of molecular changes that accompany CVD risk reduction during lifestyle change are poorly understood. There is growing evidence that peripheral blood gene expression reflects the pathophysiology of circulating leukocytes and the vascular endothelium. An increased understanding of dynamic changes in the leukocyte transcriptome during lifestyle modification thus may be crucial for evaluating the efficacy of risk-reduction strategies and understanding mechanisms by which diet and exercise affect cellular processes involved in CVD risk reduction. Conventional risk factors such as low-density lipoprotein cholesterol and blood pressure continue to be primary targets of clinical management for patients with CVD, but as new biochemical and genomic risk factors are identified, it is becoming clear that measures of vascular health go beyond traditional risk factors. A key finding of this study is that successful, sustained modulation and dramatic downregulation of genes, including LTF, through healthy changes in lifestyle may have positive effects on vascular health not readily apparent from traditional risk factors. Future studies are needed to validate changes in gene expression during lifestyle modification and examine the effect of healthy behaviors on leukocyte function and leukocyte–endothelium interactions that are important for cardiovascular health.

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Disclosures

None.

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CLINICAL PERSPECTIVE

Lifestyle interventions designed to reverse or stabilize progression of coronary artery disease successfully ameliorate clinically relevant risk factors important in the pathogenesis of atherosclerosis, but little is known about molecular alterations that accompany lifestyle changes. This study examined the effect of a rigorous cardiovascular risk-reduction program on peripheral blood gene expression profiles to characterize molecular responses and identify regulatory pathways important to cardiovascular health. During intensive lifestyle modification, expression of numerous individual genes and multigene pathways associated with leukocyte function, vascular inflammation, and lipid homeostasis were significantly downregulated. Similar to traditional risk factors, however, changes in the leukocyte transcriptome were dynamic, and persistence over time may depend on long-term adherence to healthy behaviors. As growing evidence suggests that peripheral blood gene expression reflects the pathophysiology of circulating leukocytes and health of the vascular endothelium, successful and sustained modulation of gene expression through changes in lifestyle may have beneficial effects on the vascular system of cardiac patients not apparent from traditional risk factors. Monitoring gene expression is, therefore, potentially useful for determining the vascular benefits of clinical interventions and may identify important targets for drug development.

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ORIGINAL ARTICLE

Fatigued on Venus, sleepy on Mars—gender and racial differences in symptoms of sleep apnea

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Abstract
Objective Clinical guidelines for the care of obstructive sleep apnea (OSA) recommend evaluation of daytime sleepiness but do not specify evaluation of fatigue. We studied how subjects with and without OSA experience fatigue and sleepiness, examining the role of gender and race.
Design, setting, patients Consecutive subjects entering our heart health registry completed validated questionnaires including Berlin Questionnaire for OSA, Fatigue Scale, and Epworth Sleepiness Scale. Data analysis was performed only with Whites and Blacks as there were too few subjects of other races for comparison.
Results Of 384 consecutive subjects, including 218 women (57 %), there were 230 Whites (60 %) and 154 Blacks (40 %), with average age of 55.9±12.8 years. Berlin Questionnaires identified 221 subjects (58 %) as having high likelihood for OSA. Fatigue was much more common in women (75 %) than in men (46 %) with OSA ($p<0.001$), while frequency of fatigue was similar in women (30 %) and men (29 %) without OSA ($p=0.86$). In multivariate analysis, men with OSA were sleepier than women; Black men with OSA had higher Epworth scores (mean±SD, 12.8±5.2) compared to White men (10.6±5.3), White women (10.0±4.5), and Black women (10.5±5.2), $p=0.05$. These gender differences were not related to the effects of age, body mass index, perceived stress, sleep duration, or thyroid function.
Conclusions Women report fatigue more commonly with OSA than men. Men experience sleepiness more commonly with OSA than women. The findings suggest that evaluation of sleep disorders must include an assessment of fatigue in addition to sleepiness to capture the experience of women.

Keywords Sleepiness · Fatigue · Obstructive sleep apnea syndrome · Sleep apnea

Abbreviations

BMI	Body mass index
CMS	Centers for Medicare and Medicaid Services
CPAP	Continuous positive airway pressure
EDS	Excessive daytime somnolence
ESS	Epworth sleepiness scale
ICHP	Integrative Cardiac Health Project
IRB	Institutional Review Board
OSA	Obstructive sleep apnea
OSAS	Obstructive sleep apnea syndrome
PSS	Perceived stress scale
SD	Standard deviation
TSH	Thyroid-stimulating hormone

Introduction

Obstructive sleep apnea (OSA) is an important disorder because of its high prevalence [1], the constellation of comorbidities associated with the disorder [2], and the substantial symptoms that OSA may cause [3]. OSA is labeled

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obstructive sleep apnea syndrome (OSAS) when adequate numbers of apneas and hypopneas are accompanied by symptoms such as excessive daytime sleepiness (EDS), fatigue, inattentiveness, moodiness, or morning headaches [4].

In addition to their role in diagnosis of the syndrome, symptoms also serve as important indicators to track response to therapy. A recently published clinical guideline for evaluation and management of OSA [5] endorses the evaluation of sleepiness with the Epworth Sleepiness Scale (ESS) [6] but does not suggest an assessment of fatigue. Other recently published research demonstrates that the ESS is commonly used to evaluate OSA-associated symptoms without incorporation of a scale to measure fatigue [7, 8]. However, subjects with OSA more frequently use terms such as fatigue, tiredness, or lack of energy rather than sleepiness to characterize their symptoms pointing to a lack of connection between the questions asked to elicit symptoms and the experience of symptoms by patients with OSA [9, 10].

Furthermore, symptoms of OSAS are not experienced to the same degree by patients with similar severities of OSA as measured by apnea–hypopnea index or oxygen desaturation [9, 11]. The range and severity of symptoms caused by the sleep disruption of OSA appear to be trait-like qualities for an individual patient [12, 13] and differ markedly among individuals [11]. Substantial data support the contention that sleepiness and fatigue are independent manifestations of sleep disorders and that patients may report one or the other, both or neither while carrying the same objective diagnosis of OSA [9, 10, 14, 15]. While prior research has examined gender differences in symptoms of OSAS [9, 15], we sought to broaden our understanding of the experience of sleepiness and fatigue in subjects with and without OSA with special attention to the roles of gender and race. Such an evaluation has not been previously undertaken.

Methods

This study was conducted in accordance with the amended Declaration of Helsinki and with the approval of the Institutional Review Board (IRB) at the Walter Reed National Military Medical Center in Bethesda, Maryland, which granted approval for the protocol designated #372910. The study design is an analysis of data prospectively collected on consecutive patients enrolled in the Integrative Cardiac Health Project (ICHP) Registry. The ICHP Registry is a cardiovascular disease prevention program operating in a research Center of Excellence for the United States Department of Defense. Because the Registry database could be de-identified before data analysis, an exempt protocol was approved by the IRB (#20012) to perform a secondary analysis on the Registry data and patient consent was not required for the purpose of this analysis.

Patients are self-referred or referred to the ICHP Registry by a health care provider to improve habits of diet, exercise, sleep, and stress management. ICHP is accessible to military health care beneficiaries including active duty service members, retirees, and civilian dependents. The program therefore enrolls a broad spectrum of subjects including a variety of races and ethnic backgrounds, both genders, and a range of ages from 18 to 90 years. The typical patient entering the program is found to have two to four risk factors for cardiovascular disease.

Upon entry, subjects are asked to complete a series of questionnaires (described in detail below) to gather information on demographics, current symptoms, and lifestyle habits. Among the questionnaires are validated surveys to assess sleep behaviors, sleep quality, and daytime symptoms. Data from the questionnaires are reviewed during a medical interview with a nurse practitioner who performs a physical examination with anthropomorphic measures. Patients also submit blood for laboratory tests including a thyroid function panel.

Berlin questionnaire

Of questionnaires available to screen patients for sleep apnea, the Berlin Questionnaire is one of the most commonly utilized and best validated [16]. Permission was granted by the copyright owner to use the questionnaire for this study. As measured by the questionnaire, patients with persistent and frequent signs and symptoms are considered to be at high risk for sleep apnea. Questions about symptoms demonstrated internal consistency (Cronbach correlations, 0.86 to 0.92). With a positive Berlin questionnaire, sleep apnea was predicted with a sensitivity of 0.86, a specificity of 0.77, a positive predictive value of 0.89, and a likelihood ratio of 3.79.

Fatigue Scale

The Fatigue Scale is borrowed from the Stanford Patient Education Research Center [17]. The Stanford web site stipulates that the scale is free to use without permission. The Fatigue Scale asks subjects to express their experience of fatigue from 0 to 10 for the previous 2-week period. The Fatigue Scale was tested on 122 subjects deriving a data set with mean score of 4.89±2.71 points. Subjects who circle 5 to 6 express mild fatigue, 7 to 8 moderate fatigue, and 9 to 10 severe fatigue.

Epworth sleepiness scale

The ESS is the most widely used tool to estimate the subjective symptom of daytime sleepiness [18]. Dr. Johns permits use of the ESS by individual people (including clinicians and researchers) free of charge. Subjects are asked to use a scale of 0 to 3 to estimate their likelihood of dozing in eight different

situations in recent weeks. The individual scores are summed and possible scores range from 0 to 24. Sleepy subjects score 11 or higher and sleepiness can be categorized by scores: 11 to 14, mild sleepiness; 15 to 19, moderate sleepiness; and 20 to 24, severe sleepiness.

Perceived stress scale

The perceived stress scale (PSS) is one of the most widely accepted measures of stress [19]. Dr. Cohen's web site, where a copy of the PSS is provided, states that permission for use of the scale is not necessary when use is for academic research or educational purposes. This validated 14-item questionnaire asks the subject how often certain experiences of stress occurred in the last month and is designed to measure the degree to which situations in one's life are appraised as stressful. With item responses from 0 to 4, the range of possible scores is 0 to 56 with higher scores correlating with higher stress. The PSS is designed for use in community samples with at least a junior high school education. The items are easy to understand and the response alternatives are simple to grasp. Moreover, the questions are quite general in nature and hence relatively free of content specific to any subpopulation group. Score in the low 20s reveal moderate stress levels while scores approaching 30 are substantial and concerning.

Statistical analysis

Continuous data that were normally distributed (as determined by the Shapiro–Wilk test) are presented using means with standard deviations (mean±SD): Univariate comparisons are made using the two-sample *t* test or analysis of variance. Categorical data are presented as counts with proportions and groups are compared using Fisher's exact test. Sleepiness was defined as a score on the ESS of 11 or higher, and fatigue was defined as a score on the Fatigue Scale of 5 or higher.

To adjust for confounding variables, multivariable linear regression was used with either the Fatigue Scale or ESS as the dependent variable and independent variables to include gender, race, age, body mass index (BMI), PSS, thyroid-stimulating hormone (TSH), and sleep duration. Separate models were examined for subjects with and without OSA. Independent variables that were significant in univariate analysis at the $p<0.25$ level were entered into the multivariable models [20]. Data were analyzed using IBM SPSS Statistics for Windows (v. 21.0. IBM Corp. Armonk, NY).

Results

The ICHP Registry enrolled 446 participants. The mean age± standard deviation (SD) of the participants was 55.0± 12.8 years consistent with a spectrum of lifestyles from actively working to semi-retired to fully retired adults. Of the 446 consecutive subjects, 249 women (56 %), there were 234 Whites, 155 Blacks, 13 Hispanics, 2 Asians, and 42 others. Because there were so few participants represented by racial categories other than Whites and Blacks, the other races were not considered further, leaving 389 subjects. Five subjects did not have Epworth or Fatigue Scale data leaving 384 evaluable subjects with an average age of 55.9±12.8 years and including 218 women (57 %).

Fatigue was found in 181 subjects (48 %) and sleepiness in 160 subjects (42 %). The proportion of subjects reporting neither fatigue nor sleepiness, fatigue only, sleepiness only, or both fatigue and sleepiness are shown in Table 1 by race and gender. Women had higher Fatigue Scale scores (Table 2, $p=0.02$), and complained more frequently of fatigue (115 of 215, 53 %) than men (66 of 165, 40 %), while men had significantly higher Epworth scores (Table 3, $p=0.02$), and complained more frequently of sleepiness (77 of 166, 46 %) compared to women (83 of 218, 38 %).

Berlin Questionnaires identified 219 subjects (58 %) as having high likelihood for OSA. There was no difference in thyroid function between subjects with and without a positive Berlin score (mean±SD in each group was 2.2±1.4, $p=0.61$). Symptoms of fatigue and sleepiness are presented in Figs. 1 and 2. Fatigue associated with OSA is more commonly experienced by women than by men, $p<0.001$ (Table 2 and Fig. 1). Sleepiness in association with OSA is more frequently experienced by men, particularly Black men, than by all other categories, $p=0.05$ (Table 3 and Fig. 2).

Univariate analysis of Fatigue Scale scores (Table 2) demonstrates significantly higher scores in younger age groups ($p<0.001$), and in subjects with positive Berlin score ($p<0.001$), higher perceived stress scores ($p<0.001$), and shorter sleep duration ($p<0.001$). Notably, Fatigue Scale scores were not different according to TSH, nor were they different according to BMI categories after factoring in presence of OSA (Table 2).

Univariate analysis of ESS scores (Table 3) show higher scores in younger age categories ($p<0.001$), and in subjects with positive Berlin scores ($p<0.001$), higher perceived stress scores ($p<0.001$), and shorter sleep duration ($p<0.001$). ESS scores were not different according to TSH, nor were they different according to BMI categories after factoring in presence of OSA (Table 3).

To control for confounding demographic and clinical characteristics, multivariable linear regression was used to examine both fatigue and sleepiness. With the Fatigue Scale score as the dependent variable, age and perceived stress score both significantly correlated with fatigue in subjects without OSA. Younger age and higher stress were associated with more fatigue. However, among subjects with OSA, gender was also

Table 1 Symptoms by gender and race

Subject descriptors	All subjects[a] (n=380)	Black women (n=89)	White women (n=126)	Black men (n=63)	White men (n=102)	p value
Age (years)	56.0±12.8	52.9±12.0	56.9±12.0	52.1±13.6	59.9±12.9	<0.001
BMI (kg/m^2)	30.7±5.4	32.5±5.8	29.2±5.3	31.2±4.6	30.7±5.1	<0.001
Not fatigued, not sleepy	141 (37 %)	23 (26 %)	54 (43 %)	25 (40 %)	39 (38 %)	0.007
Fatigued only	81 (21 %)	28 (31 %)	29 (23 %)	6 (9 %)	18 (18 %)	
Sleepy only	58 (15 %)	9 (10 %)	14 (11 %)	15 (24 %)	20 (20 %)	
Both fatigued and sleepy	100 (26 %)	29 (33 %)	29 (23 %)	17 (27 %)	25 (24 %)	

Age, BMI, and the proportion of subjects reporting neither fatigue nor sleepiness, fatigue only, sleepiness only, or both fatigue and sleepiness are shown by race and gender. For age and BMI, comparisons between groups are made using analysis of variance. For the categorical variables of fatigue and sleepiness, comparisons between groups are made using Fisher's exact test. Fatigue was defined as a score on the Fatigue Scale of 5 or higher, and sleepiness was defined as a score on the Epworth Sleepiness Scale of 11 or higher

[a] Three hundred eighty of the 384 subjects had both Epworth and fatigue data

significantly associated with fatigue, with women reporting higher fatigue scores compared to men (Table 4).

Multiple linear regression using ESS score as the dependent variable showed that the independent variable of sleep duration was significantly associated with sleepiness among subjects without OSA, with longer sleep times associated with lower ESS scores. However, among subjects with OSA, PSS and gender were significantly associated with ESS scores. Increases in perceived stress were associated with higher levels of sleepiness. Since female gender was the reference group in the model, the positive beta coefficient for gender indicates a greater degree of sleepiness in men compared to women (Table 5).

Table 2 Fatigue scale data compared for subjects with and without OSA

Fatigue scale		Total			No OSA			OSA		
		n	mean±SD	p value	n	mean±SD	p value	n	mean±SD	p value
All subjects		380	4.4±2.4		161	3.4±2.2		219	5.1±2.3	
Gender	Females	215	4.7±2.5	0.022	105	3.5±2.3	0.58	110	5.8±2.2	<0.001
	Males	165	4.1±2.3		56	3.3±2.1		109	4.5±2.3	
Race	Black	152	4.8±2.4	0.028	56	3.6±2.3	0.54	96	5.4±2.3	0.1
	White	228	4.2±2.4		105	3.4±2.1		123	4.9±2.4	
Gender × race	Black females	89	5.3±2.5	0.002	33	4.0±2.4	0.26	56	6.0±2.3	<0.001
	White females	126	4.2±2.4		72	3.3±2.1		54	5.5±2.2	
	Black males	63	4.0±2.1		23	3.0±1.9		40	4.6±2.0	
	White males	102	4.1±2.4		33	3.6±2.2		69	4.4±2.4	
Age (years)	<50	106	5.6±2.0	<0.001	39	4.6±2.0	<0.001	67	6.2±1.8	<0.001
	50-59	131	4.5±2.5		48	3.6±2.3		83	5.0±2.5	
	60+	143	3.5±2.2		74	2.8±1.9		69	4.3±2.3	
BMI	Normal	51	4.4±2.7	0.02	31	3.5±2.5	0.61	20	5.8±2.6	0.47
	Overweight	129	4.0±2.4		78	3.3±2.2		51	5.0±2.5	
	Obese	200	4.7±2.3		52	3.6±2.1		148	4.3±2.3	
Berlin questionnaire	Normal	161	3.4±2.2	<0.001	161	3.4±2.2				
	OSA	219	5.1±2.4					219	5.1±2.4	
TSH (mU/L)	<4.5	361	4.4±2.4	0.29	154	3.4±2.2	0.3	207	5.1±2.4	0.68
	4.5 +	19	5.0±2.2		7	4.3±1.6		12	5.4±2.4	
PSS (of 56 points)	<21	176	3.4±2.3	<0.001	92	2.7±2.0	<0.001	84	4.1±2.4	<0.001
	21+	200	5.3±2.2		69	4.4±2.1		131	5.8±2.1	
Sleep duration (h)	<6	120	5.4±2.3	<0.001	37	4.3±2.5	0.005	83	5.8±2.1	<0.001
	6+	257	4.0±2.4		122	3.2±2.1		135	4.7±2.4	

Fatigue scale data are presented according to various categories listed on the left column of the table. Comparisons between groups are made using the two-sample t test or analysis of variance

Table 3 Epworth score data compared for subjects with and without OSA

Epworth score		Total			No OSA			OSA		
		n	mean±SD	*p* value	*n*	mean±SD	*p* value	*n*	mean±SD	*p* value
All subjects		384	9.4±5.2		163	7.5±4.7		221	10.9±5.1	
Gender	Females	218	8.9±5.0	0.024	106	7.4±4.8	0.87	112	10.3±4.9	0.096
	Males	166	10.1±5.3		57	7.5±4.4		109	11.4±5.3	
Race	Black	154	10.4±5.4	0.002	57	8.7±5.1	0.015	97	11.5±5.3	0.11
	White	230	8.7±4.9		106	6.8±4.3		124	10.4±4.9	
Gender × race	Black females	91	9.9±5.2	0.001	34	8.9±5.0	0.11	57	10.5±5.2	0.05
	White females	127	8.2±4.8		72	6.7±4.6		55	10.0±4.5	
	Black males	63	11.2±5.6		23	8.4±5.4		40	12.8±5.2	
	White males	103	9.4±5.1		34	7.0±3.5		69	10.6±5.3	
Age (years)	<50	108	11.2±5.5	<0.001	41	8.8±5.3	0.038	67	12.7±5.1	0.002
	50–59	133	9.3±4.9		48	7.8±4.3		85	10.1±5.0	
	60+	143	8.2±4.8		74	6.5±4.3		69	10.0±4.8	
BMI (kg/m^2)	Normal	51	8.7±5.7	0.056	31	6.2±4.7	0.09	20	12.6±5.1	0.26
	Overweight	131	8.8±5.4		79	7.3±4.8		52	11.0±5.5	
	Obese	202	10.0±4.9		53	8.5±4.4		149	10.6±4.9	
Berlin questionnaire	Normal	163	7.5±4.7	<0.001	163	7.5±4.7				
	OSA	221	10.9±5.1					221	10.9±5.1	
TSH (mU/L)	<4.5	365	9.3±5.2	0.19	156	7.3±4.7	0.1	209	10.8±5.2	0.74
	4.5 +	19	10.9±4.3		7	10.3±4.3		12	11.3±4.4	
PSS (of 56 points)	<21	177	8.2±4.7	<0.001	93	7.3±4.6	0.55	84	9.1±4.7	<0.001
	21+	203	10.4±5.3		70	7.7±4.7		133	11.8±5.1	
Sleep duration (h)	<6	121	11.0±5.5	<0.001	37	9.2±5.5	0.023	84	11.7±5.3	0.051
	6+	260	8.7±4.9		124	6.9±4.3		136	10.3±4.9	

Epworth Sleepiness Scale data are presented according to various categories listed on the left column of the table. Comparisons between groups are made using the two-sample *t* test or analysis of variance

Discussion

The salient findings of this study are that symptoms of sleepiness and fatigue experienced in association with OSA have different frequencies by gender and by race even after controlling for confounding variables such as age, BMI, thyroid function, and self-reported total sleep time. In particular, gender was the most strongly predictive variable. These findings are of obvious importance to clinicians evaluating and following subjects with OSA since patients need to be provided with the proper questionnaire tools to quantify their subjective complaints. Evaluating the symptom of fatigue with a

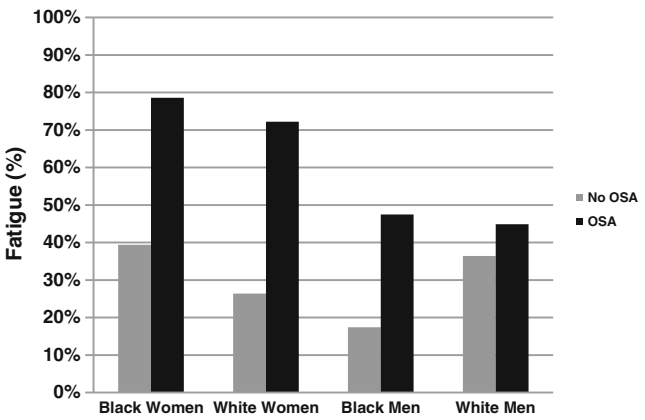

Fig. 1 Frequency of fatigue by race and gender. Fatigue associated with obstructive sleep apnea (OSA) is more commonly experienced by women than by men, $p<0.001$

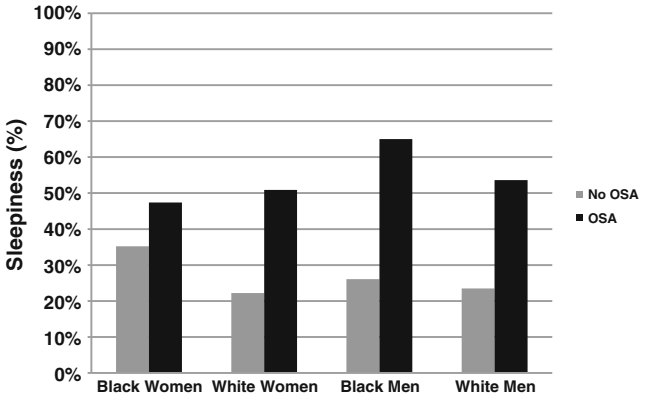

Fig. 2 Frequency of sleepiness by race and gender. Sleepiness in association with obstructive sleep apnea (OSA) is more frequently experienced by men, particularly Black men, than by all other categories, $p=0.05$

Table 4 Results of multivariate linear regression for fatigue score

Independent variables	No OSA		OSA	
	Adjusted coefficients		Adjusted coefficients	
	Beta (95 % CI)	*p* value	Beta (95 % CI)	*p* value
Age	−0.04 (−0.07 to −0.02)	<0.001	−0.03 (−0.05 to −0.004)	0.022
BMI	NS		NS	
PSS	0.09 (0.05 to 0.13)	<0.001	0.09 (0.05 to 0.12)	<0.001
Sleep duration	−0.21 (−0.45 to 0.03)	0.079	−0.14 (−0.36 to 0.09)	0.23
TSH	NS		NS	
Gender[a]	NS		−1.02 (−1.59 to 0.45)	0.001
Race[b]	NS		−0.11 (−0.72 to 0.50)	0.72

To adjust for confounding variables, multivariate linear regression was used with Fatigue Scale as the dependent variable and independent variables to include gender, race, age, BMI, PSS, TSH, and sleep duration. Separate models were examined for subjects with and without OSA. Independent variables that were significant in univariate analysis at the $p<0.25$ level were entered into the multivariate models. NS indicates that a variable was not significant in univariate analysis and was therefore not included in the multivariate model

BMI body mass index, *OSA* obstructive sleep apnea, *PSS* perceived stress scale, *TSH* thyroid-stimulating hormone

[a] Females are reference group

[b] Blacks are reference group

questionnaire designed to quantify sleepiness will not suffice. Likewise, sleepiness cannot be properly evaluated with a questionnaire aimed at the symptom of fatigue. It is of major interest that a sizable proportion of the study subjects (10 to 31 % according to gender and race) experienced fatigue without sleepiness.

The proper documentation of symptoms is also important to gain appropriate allowance by insurance carriers. The National Coverage Determination for continuous positive airway pressure (CPAP) therapy published by the Centers for Medicare and Medicaid Services (CMS) sets the standard for Medicare coverage and is adopted by other insurance providers [21]. CMS considers CPAP therapy reasonable and necessary for patients with a mild category of OSA (apnea hypopnea index or respiratory disturbance index greater than or equal to five events and less than or equal to 14 events per hour) if appropriate symptoms are documented [21]. Without symptoms properly documented in these patients with a mild index of severity, their CPAP therapy would not be justifiable to insurance carriers, including CMS.

Table 5 Results of multivariate linear regression for Epworth sleepiness score

Independent variables	No OSA		OSA	
	Adjusted coefficients		Adjusted coefficients	
	Beta (95 % CI)	*p* value	Beta (95 % CI)	*p* value
Age	−0.04 (−0.09 to 0.01)	0.15	−0.03 (−0.09 to 0.03)	0.28
BMI	0.10 (−0.06 to 0.26)	0.20	NS	
PSS	NS		0.17 (0.08 to 0.25)	<0.001
Sleep duration	−0.71 (−1.27 to −0.16)	0.012	−0.19 (−0.71 to 0.33)	0.47
TSH	0.31 (−0.22 to 0.84)	0.25	NS	
Gender[a]	NS		1.59 (0.27 to 2.90)	0.018
Race[b]	−1.30 (−2.89 to 0.29)	0.11	−0.97 (−2.37 to 0.43)	0.17

To adjust for confounding variables, multivariate linear regression was used with Epworth Sleepiness Scale as the dependent variable and independent variables to include gender, race, age, BMI, PSS, TSH, and sleep duration. Separate models were examined for subjects with and without OSA. Independent variables that were significant in univariate analysis at the $p<0.25$ level were entered into the multivariate models. NS indicates that a variable was not significant in univariate analysis and was therefore not included in the multivariate model

BMI body mass index, *OSA* obstructive sleep apnea, *PSS* perceived stress scale, *TSH* thyroid-stimulating hormone

[a] Females are reference group

[b] Blacks are reference group

The finding of increased sleepiness and fatigue with shorter sleep duration conforms to prior studies that have demonstrated a strong correlation of acute and chronic sleep deprivation with decreased alertness, impaired psychomotor vigilance testing, and shorter sleep latency on mean sleep latency test [22–24]. Likewise, the observation that sleepiness and fatigue decrease with higher age groups agrees with prior research [25, 34]. We speculate that this finding of diminished symptoms with age is further explained by the circumstances that retirement and semi-retirement in older age groups allows for more opportunities to sleep and to sleep on a self-determined schedule.

The association of higher stress levels with increased symptoms of fatigue and sleepiness deserves to be addressed with further scrutiny. Potential explanations are that higher perceived stress levels intensify the experience of other symptoms such as fatigue and sleepiness. It is equally plausible that high stress levels negatively affect sleep latency, sleep continuity, and the restorative quality of sleep. These theoretical considerations warrant further study and suggest that successful stress management may be an intervention as valuable as expansion of sleep time for symptom management.

The findings of a differential experience of symptoms from disturbed sleep according to gender and race are not unique to this study. Recent reports include the observations that women more frequently experience sleep-onset insomnia than men [26] and that White women are more likely to report use of a sleep aid (prescription or nonprescription) [27]. Periodic limb movements of sleep and associated symptoms are much more common in Whites compared to Blacks [28], while estimated prevalence of narcolepsy and its symptoms are higher in women than men and in Blacks than in other racial groups [29]. Blacks are more likely to experience sleep phase advance [30] and both Blacks and women are more likely to report extremes of sleep duration (less than 5 h or greater than 9 h) [31, 32] with attendant elevation in C-reactive protein [33].

In a published review of gender differences, Ye and colleagues raise the concern that differences in symptoms on presentation with OSA may lead to the under-recognition of sleep pathology in women [15]. They note that while the Sleep Heart Health Study [34] did not find the frequency or severity of sleepiness to be affected by gender, the Wisconsin Sleep Cohort Study [1] did report a higher proportion of women with daytime sleepiness than men. Data from the Sleep Heart Health Study analyzed for impact of ethnicity but not gender [35] did find less subjective sleepiness among Blacks than Whites. Other studies report that men tend to report more sleepiness than women [36], and that women prefer to describe their subjective experience of sleep-disordered breathing using terms to denote fatigue, tiredness, and lack of energy [9, 18]. One explanation for these disparate findings regarding the different experiences of symptoms is that the questionnaire instruments may not have allowed participants, especially women, the chance to register symptoms of fatigue.

Research into the differential experience of the subjective symptoms of sleepiness versus fatigue is acknowledged to be difficult [37] and a variety of potential explanations for the disparate published reports above have been advanced. Among the explanations are that men have a less accurate perception of their pathologies than do women, that cultural influences make men less willing to acknowledge symptoms, or that there may be a gender-based neurophysiological explanation for the different experience of OSA [9]. Explanations of racial differences include the impact of socioeconomic conditions [8, 38] and varied subjective interpretation of symptoms due to differing life experiences [39]. However, there are studies that demonstrate clear anatomical differences of the upper airway according to gender and race [40]. Furthermore, a gene association study [41] and gene segregation analysis [42] have documented associations of sleep apnea vulnerability according to race.

A limitation of the current study is that subjects were categorized for the presence of sleep apnea using the Berlin Questionnaire rather than polysomnography. The Berlin Questionnaire is a reasonably sensitive and specific clinical screening tool but it is not the gold standard, suggesting that an appropriate follow-on study may be to repeat our measures in a large population with polysomnography. Another limitation is that races other than Whites and Blacks were not represented in sufficient numbers to include them in this analysis. The symptoms experienced by men and women of other races deserve further discovery.

Another factor potentially limits the ability to generalize our findings to other populations. A third of the subjects in our study sample reported fewer than 6 h of sleep per night. This degree of sleep restriction is higher than that reported in civilian populations and may be a reflection of the military culture from which our study sample derives [43]. A survey of the average sleep duration in the USA reported in 2009 that approximately 40 % of military personnel obtained less than 5 h of sleep per night compared with 8 % in the general population [43].

The data from the current study indicate that the subjective symptoms of sleepiness and fatigue are experienced not just according to gender or race but differentially by both factors simultaneously. These findings underscore the clear need to evaluate patients presenting with sleep disorders using instruments that measure more than just sleepiness and incorporate measures of fatigue and other descriptors commonly voiced by patients suffering from sleep conditions. Clinical centers evaluating patients for sleep disorders would be well advised to incorporate validated instruments for assessing symptoms of fatigue in addition to sleepiness. Future clinical guidelines should incorporate the recommendation that the evaluation of patients with sleep complaints include assessment of symptoms such as fatigue.

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A Systematic Approach Incorporating Family History Improves Identification of Cardiovascular Disease Risk

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Background: Although family history (FH) is an independent predictor of cardiovascular disease (CVD) risk, traditional risk scores do not incorporate FH. Nurse practitioners routinely solicit FH but have no mechanism to incorporate the information into risk estimation. Underestimation of risk leaves clinicians misinformed and patients vulnerable to the CVD epidemic. **Objective:** We examined a systematic approach incorporating FH in CVD risk assessment, validating risk reclassification using carotid intima-media thickness (CIMT), a surrogate measure of atherosclerosis. **Methods:** Of 413 consecutive patients prospectively enrolled in the Integrative Cardiac Health Project Registry, a subgroup of 239 was low or intermediate risk by the Framingham Risk Score. A systematic approach for the assessment of FH was applied to this subgroup of the registry. A positive FH for premature CVD, defined as a first-degree relative having a CVD event before the age of 55 years in men and 65 years in women, conferred reclassification to high risk. Reclassification was validated with CIMT results. **Results:** Chart audits revealed adherence to the systematic approach for FH assessment in 100% of cases. This systematic approach identified 115 of 239 (48%) patients as high risk because of positive FH. Of the reclassified patients, 75% had evidence of subclinical atherosclerosis by CIMT versus 55% in the patients not reclassified, $P < 0.001$. Logistic regression identified positive FH for premature CVD (odds ratio, 2.6; $P = 0.001$) among all variables, as the most significant predictor of abnormal CIMT, thus increasing risk for CVD. **Conclusions:** The Integrative Cardiac Health Project systematic approach incorporating FH into risk stratification enhances CVD risk assessment by identifying previously unrecognized high-risk patients, reduces variability in practice, and appropriately targets more stringent therapeutic goals for prevention.

KEY WORDS: cardiovascular disease, family history, primary prevention, risk assessment

Cardiovascular disease (CVD) is the leading cause of death and disability in the United States and Europe.[1,2] On the basis of numerous analyses performed to determine the thresholds for increased risk, family history (FH) of premature CVD is defined as a first-degree relative having a CVD event before the age of 55 years in men and 65 years in women.[3–12] With this definition, FH of premature CVD is an independent and robust predictor of risk. When FH is positive, individual risk for CVD is increased by as much as 5-fold.[10] Although US and European guidelines include positive FH as a high-risk factor, traditional risk scoring systems do not. Nurse practitioners routinely inquire about FH in clinical practice, but there is variability in the approach to capture and interpret the data.[5,13,14]

The Framingham Risk Score (FRS), the most widely used CVD risk assessment tool, significantly underestimates risk because it does not incorporate FH data.[15,16] Studies show FRS to be only 50% accurate in identifying patients at high risk for heart disease.[15] In fact, up to 75% of patients experiencing an acute coronary syndrome are assessed as low risk by the FRS.[17] When FH is not used in risk assessment, a large subgroup of the population at risk for CVD remains unrecognized, leaving them unaware of their threatened health status. Failing

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to identify these high-risk individuals precludes clinicians from prescribing targeted and risk-specific self-care interventions aimed at CVD prevention.[13]

Although FH has been repeatedly demonstrated to be a high risk factor of CVD, current guidelines provide no mechanism for the systematic collection, interpretation, and risk score adjustment using this information. We implemented a systematic approach for the assessment of FH to standardize identification of high-risk patients and used carotid intima-media thickness (CIMT) to validate the high-risk reclassification.[18,19]

Methods

This investigation was conducted with the approval of the institutional review board at Walter Reed National Military Medical Center in Bethesda, Maryland. The study design is a subgroup analysis of data prospectively collected on consecutive patients enrolled in the Integrative Cardiac Health Project (ICHP) Registry. The ICHP Registry is a CVD prevention program operating in a research Center of Excellence for the US Department of Defense. All subjects gave informed consent for participation in the registry, and the study was conducted according to the principles stated in the Declaration of Helsinki.

The ICHP offers military healthcare beneficiaries a 6-month tailored CVD risk reduction program. Patients who join the program by self or provider referral must be adults older than 17 years. All patients seen at the ICHP are categorized upon baseline assessment as low, intermediate, or high risk for CVD by the FRS. In addition, ICHP patients receive results of a detailed CVD risk assessment and a personalized preventive health plan. As part of the ICHP Registry, patients receive a CIMT, which is maintained as a long-term CVD outcome measure. The CIMT findings are not used to calculate the patient's CVD risk status. The following variables were collected on all patients who attended the ICHP from 2008 to 2011: age, gender, ethnicity, FRS, FH status, CIMT and diagnoses of CVD, hypertension, dyslipidemia, and diabetes.

Upon entry to the ICHP, patients undergo a cardiovascular-focused history and physical examination. Medical history, including smoking history, is elicited with a written question as part of a questionnaire, and the responses are verified verbally by a nurse practitioner at the time of the physical examination. medical history such as hypertension, diabetes, and dyslipidemia is also elicited on the questionnaire, validated verbally by a nurse practitioner and reconciled with data recorded in the patient's medical record. Body mass index (BMI) is calculated with the formula kilograms divided by the square of height in meters using measured height and weight from a medical-grade weight scale and stadiometer. Blood pressure is first measured after the patient has been sitting quietly for 5 minutes using a GE DINAMAP PRO Series 100–400V2. Five minutes later, a second blood pressure reading is taken, and the 2 values are averaged for the record. All cardiovascular-relevant laboratory data are obtained in the blood chemistry laboratory at the medical facility, with the laboratory certified by the Clinical Laboratory Improvement Amendments.

At a subsequent appointment, the patients were informed of their CVD risk status and were provided therapeutic goals specific to their determined risk category. Although the patients in all risk categories (low, intermediate, and high) received recommendations for healthy behavior change, the high-risk patients were targeted with aggressive treatment goals for cholesterol, blood pressure, and weight management.

This analysis was limited to a subgroup of ICHP patients whose calculated FRS showed low or intermediate 10-year risk because the high-risk patients could not be reclassified to a higher level of risk. Diabetes is considered by the FRS to be a high-risk factor, and therefore, any patient with diabetes was excluded from this analysis.

Risk Assessment (Carotid Intima-Media Thickness)

The CIMT findings were reviewed and evaluated by 1 sonographer oriented to the purposes of the project but blinded to the FH information for each patient. Images were obtained on a single ultrasound machine (SonoSite MicroMaxx 3.4.3; Bothell, Washington) using a linear array 5- to 10-MHz transducer with standardized image settings, including resolution mode, depth of field, gain, and transmit focus. All sonograms were obtained with the patients supine with the head facing the contralateral side. Electrocardiograms were recorded simultaneously. The sonographer, also trained in the measurement of CIMT, performed the analyses with commercially available software (Sonocalc IMT, Bothell, Washington). Carotid intima-media thickness was determined from images of the far wall of the distal common carotid arteries (immediately proximal to the carotid bulb) and reported as the mean value for the bilateral measurement. The near (intimal-luminal interface) and far (medial-adventitial interface) field arterial wall borders were manually traced for measurement of mean CIMT (millimeters) across a 10-mm arterial segment. A mean CIMT measurement of greater than the 75th percentile cutoff value, based on age and gender, in at least 1 carotid vessel was defined as an abnormal CIMT, as proposed by the American Society of Echocardiography Carotid Intima-Media Thickness Task Force.[20] This cutoff value has been used in a prior large atherosclerosis outcomes study, the Arterial Biology for the Investigation of the Treatment Effects of Reducing Cholesterol (ARBITER) Study, with CIMT as its main outcome measure.[21]

Impact Assessment

For CVD risk assessment, ICHP nurse practitioners evaluated FRS and FH status. The FRS, which takes into account age, gender, smoking, systolic blood pressure, total cholesterol, and high-density cholesterol levels, was determined using a web-based tool.[22] A systematic approach to evaluating FH was applied to standardize risk stratification beyond the FRS (see Figure). The ICHP nurse practitioners were trained using a standardized operating procedure (SOP) detailing the collection of FH during the initial assessment of each patient. This SOP defined positive FH of premature CVD as a first-degree relative (parent or sibling) having a CVD event before the age of 55 years in men and 65 years in women.[11,12] Cardiovascular disease events included myocardial infarction; cardiovascular revascularization; and diagnosis of coronary disease, stroke, or transient ischemic attack. The family tree was explored in detail for these CVD events, specifically in first-degree relatives and for the age of occurrence. Any first-degree family member meeting these criteria conferred a high-risk designation irrespective of the FRS result. Patients who were unable to provide FH (for example, patients who are adopted and do not have FH information) were excluded from the analysis. Chart audits were performed on 100% of cases to verify adherence to the systematic approach outlined in the SOP.

Analyses were performed using the Statistical Package for the Social Sciences (version 20.0).[23] Descriptive and frequency statistics were presented as mean (SD) or percentage. Student *t* test for continuous variables and χ^2 analysis for categorical variables were used. Logistic regression was performed to assess the predictive impact of factors on the likelihood of a patient having an abnormal CIMT.

Results

Of 413 patients, 19 patients (4.6%) were excluded for lack of FH data, leaving 394 for this analysis. Using the FRS, 239 of 394 patients (61%) were classified as low or intermediate risk. Frequency and descriptive analyses revealed a normally distributed population by age with no missing data. Demographic findings showed a mean age of 49 years (range, 20–76); 59% were women; 51%, white; 25%, black; 6%, Hispanic; and 1%, Asian, with 17% undeclared or other. The mean body mass index was 30.5 kg/m^2. The population was characterized by hypertension (40%), dyslipidemia (71%), and smoking (2%).

Chart audits revealed adherence to the systematic approach for FH assessment in 100% of the 239 patients who were in the low or intermediate FRS category. The systematic approach identified 115 of 239 patients (48%) as having positive FH for CVD. Table 1 displays the comparison between the 2 groups (positive FH and negative

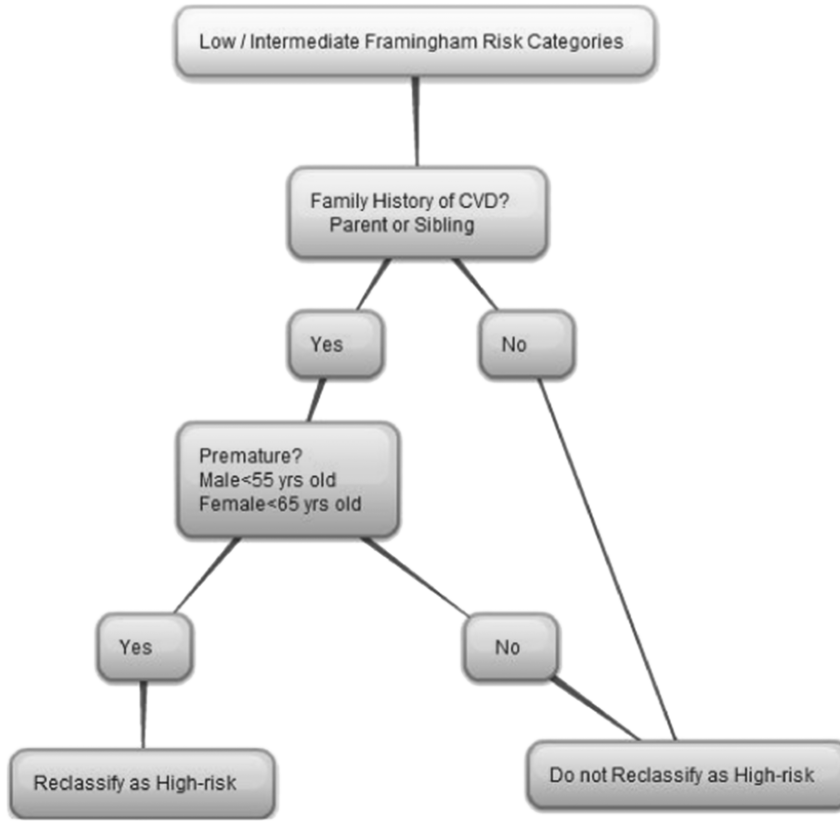

FIGURE. *The Integrative Cardiac Health Project systematic approach incorporating family history in CVD assessment.*

TABLE 1 Baseline Characteristics of Population at Low and Intermediate Cardiovascular Disease Risk

N = 239	Negative FH, n = 125	Positive FH, n = 114	*P*
Age, y	44.9 (12.18)	54.3 (10.16)	0.02[a]
Gender (female)	55%	64%	0.17
BMI, kg/m^2	29.5	31.3	0.39
Active smoker	3%	2%	0.86
Hypertension	31%	39%	0.18
Dyslipidemia	74%	70%	0.47
FRS	3.01 (3.21)	4.5 (4.19)	0.001[a]
Glucose, mg/dL	89.8 (10.1)	92.8 (9.68)	0.84
CIMT (abnormal)	55%	75%	<0.001[a]

Data are presented as mean (SD) or percentage. *t* test is used for continuous variables. χ^2 analysis is used for categorical variables. *P* values are given for the comparison between FH groups.
[a]Denotes statistical significance.

FH). Between FH groups, age, FRS, and CIMT were different. The patients with a positive FH were older (54.3 vs 44.9 years, $P = 0.02$). The mean FRS scores were statistically different (positive FH, 4.5; negative FH, 3.0; $P < 0.001$), although this difference is not clinically important because both scores indicate low risk. In validating the reclassification using CIMT, the proportion of patients with an abnormal CIMT was clinically and statistically different between groups, with a higher percentage in the positive FH group (75% vs 55%, $P < 0.001$). No effect of confounding was detected because there was no difference between groups using χ^2 analysis for gender, BMI, smoking history, hypertension, and dyslipidemia.

Logistic regression was performed to assess the impact of factors on the likelihood that patients would have an abnormal CIMT (Table 2). The model contained 5 independent variables (race, gender, FH category, diagnoses of hypertension and dyslipidemia). Age was not included in the model because age is one of the normative factors used as a cutoff value in the definition of normal versus abnormal CIMT.[20] The full model containing all predictors was statistically significant, χ^2 (11, n = 239) = 41.1, $P < 0.001$, indicating that the model was able to distinguish between normal and abnormal CIMT. The model as a whole explains between 16% and 22% of the variance in CIMT status and correctly classified 69% of cases after inclusion of the predictors. Two of the independent variables made a unique statistically significant contribution to the model (black race: odds ratio [OR], 5.8; $P = 0.02$; 95% confidence interval [CI], 1.3–26.9, and presence of positive FH: OR, 2.4; $P = 0.006$; 95% CI, 1.3–4.5). In an effort to find the most parsimonious model predicting abnormal CIMT,[24] logistic regression was repeated using the 2 contributing variables, black race and presence of positive FH. This new model containing the 2 predictors was statistically significant, χ^2 (6, n = 239) = 28.6, $P < 0.001$, indicating that the model was able to distinguish between normal and abnormal CIMT. The model as a whole explains between 11% and 16% of the variance in CIMT status and correctly classified 69% of cases after inclusion of the predictors. Although black race was no longer a significant predictor in the new model, presence of positive FH remained the only significant predictor contributing to the logistic regression model (black race: OR, 0.528; $P = 0.290$; 95% CI, 0.162–1.725, and presence of positive FH: OR, 2.64; $P = 0.001$; 95% CI, 1.47–4.73). The Hosmer-Lemeshow test showed goodness of fit with a significance of 0.86.

Discussion

Although national guidelines recognize the importance of FH for CVD risk, these guidelines provide no mechanism to instruct practitioners on how to translate this FH information to a more accurate determination of risk for the individual patient.[1,2,5] In fact, there has been

TABLE 2 Logistic Regression Model

Predictors of Abnormal CIMT	*B*	SE	Wald	*df*	*P*	OR	95% CI for OR	
							Lower	Upper
Black race	1.761	0.781	5.088	1	0.024[a]	5.816	1.260	26.856
Gender	0.441	0.318	1.921	1	0.166	1.554	0.833	2.897
FH positive	0.883	0.318	7.691	1	0.006[a]	2.418	1.296	4.513
Diagnosis of hypertension	0.540	0.346	2.435	1	0.119	1.716	0.871	3.382
Diagnosis of dyslipidemia	0.196	0.347	0.320	1	0.572	1.217	0.616	2.404
Constant	−1.736	0.808	4.612	1	0.032	0.176		

The model contained 5 independent variables (race, gender, positive FH, diagnosis of hypertension, and diagnosis of dyslipidemia). The full model containing all predictors was statistically significant, χ^2 (11, n = 239) = 41.1, $P < 0.001$, indicating that the model was able to distinguish between normal and abnormal CIMT.
[a]Denotes statistical significance.

a call for evidence on the value of systematically using FH in CVD risk assessment.[5]

Investigation of FH requires a systematic approach in which there is minimized variability in assessment of risk among clinicians because there are numerous criteria needed to fulfill the definition of positive FH. These criteria are complex and require an in-depth review of the family tree including gender, relationship to the patient, and age of onset of CVD. A simple yes/no question is inadequate to provide the relevant data to illicit an accurate FH for risk estimation.[5]

Our study population of mostly overweight, late–middle-aged subjects with a variety of races is fairly typical of a population seeking medical evaluation for CVD risk estimation. One risk factor that makes our sample population stand out as different from the US population is the very low prevalence of self-reported smoking behavior (2%), which is substantially lower than US norms (19%).[25] A potential explanation for this discrepancy is that there have been initiatives for health promotion that champion smoking cessation, including a ban of smoking on site in the medical facility. Furthermore, self-referred patients seeking wellness in a CVD risk reduction program may also be less likely to smoke.

We have shown that, among asymptomatic, previously low- or intermediate-risk patients by FRS, the use of a systematic approach for the incorporation of FH resulted in identifying a substantial proportion of patients at high risk for CVD. These patients would have otherwise been told that they were not at high risk for CVD. In addition, we have demonstrated the feasibility of implementing a systematic approach for incorporating FH, an easily accessible and inexpensive data point.[26]

The validity of this reclassification was substantiated using CIMT in the positive FH group to find 75% abnormal CIMT results compared with 55% abnormality in the group with negative FH. This is consistent with findings from the Framingham Offspring Study, a large population-based cohort of families in which CVD events were validated prospectively in both parents and offspring.[11] On the basis of that study, an association was found between parental history and subclinical atherosclerosis among offspring measured by CIMT.

Our study highlights the predictive value of including FH in assessment of risk for CVD. By logistic regression, positive FH was shown to be a robust predictor, indicating that patients with presence of positive FH were more than twice as likely to have an abnormal CIMT compared with those with negative FH, when controlling for all other factors in our data set. Although positive FH was an independent predictor, other factors including age, race, gender, and diagnoses of hypertension and dyslipidemia were not predictors of an abnormal CIMT. This may be explained by an underlying atherosclerotic mechanism causing functional abnormalities in offspring of patients with premature CVD, independent of known vascular risk factors.[27–29]

The mean age of the patients with a positive FH was greater than of the patients with negative FH in our cohort. This finding may be explained by the fact that older study subjects will have older siblings who are more likely to have experienced a cardiovascular event and younger study subjects will more likely have younger siblings who have not yet developed CVD. The older sibling's event gives the older study subject a positive FH, whereas younger study subjects are more likely to have a negative FH.

The lack of a mechanism to incorporate FH in CVD risk assessment is a major gap in current practice. This article suggests a systematic approach to translate the evidence for FH into clinical practice. When patients at high risk for CVD are properly identified, they are given appropriate therapeutic goals to match their heightened risk category, and more attention is paid to healthy lifestyle behavior change. Ultimately, incorporating FH in risk assessment is a way to personalize preventive therapies aimed at combating the epidemic of CVD.

Limitations

Limitations include the use of CIMT as a surrogate measure for CVD events. However, this is a commonly used strategy to overcome expense, feasibility issues, and risk associated with radiological studies such as electron beam computerized tomography and computed tomographic angiography.[18]

Although our sample population shows some characteristics that mirror the US population generally such as overweight,[30] an important characteristic that deviates from the US population is the very low prevalence of smoking status (2%). This difference may limit our ability to generalize our findings to the population at large. Another potential limitation may be referral bias because patients with positive FH may have a heightened sense of concern regarding their CVD health before entering the program.

Furthermore, data collection did not include all individual variables thought to influence CVD, although variables necessary for FRS calculation were captured. A further limitation is that approximately 5% of our patients were unable to provide FH.

Conclusions

Translation of evidence into practice is dynamic, and mechanisms to help clinicians accomplish translation continue to evolve. Recent evidence indicates that positive FH has predictive validity.[4] This study demonstrates that a reproducible systematic approach for adding FH to current practice enhances predictive value and identifies high-risk patients who, at present, are not captured.

This report describes a mechanism that addresses a current gap in clinical practice. The findings of this report are sufficiently promising to warrant further implementation and validation in other settings, using different study designs and outcome measures.

What's New and Important

- Family history for premature CVD, defined as a first-degree relative having a CVD event before the age of 55 years in men and 65 years in women, confers a high-risk classification for CVD as validated by a surrogate marker of atherosclerosis.
- A systematic approach for incorporation of FH for premature CVD will enhance the identification of high-risk patients.
- Incorporating FH in risk assessment is a way to personalize preventive therapies aimed at combating the epidemic of CVD.

We urge practitioners to adopt a systematic approach to incorporate FH in CVD risk assessment to provide patients with more accurate risk stratification and to target preventive interventions for high-risk individuals. We believe that implementation of such a systematic approach would have a global impact on patients at risk for CVD.

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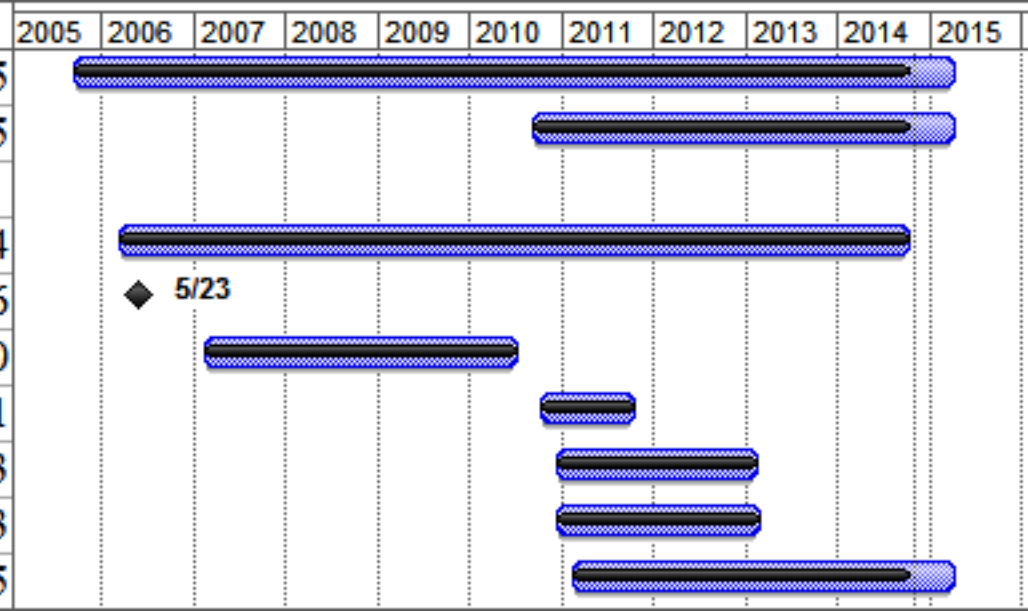
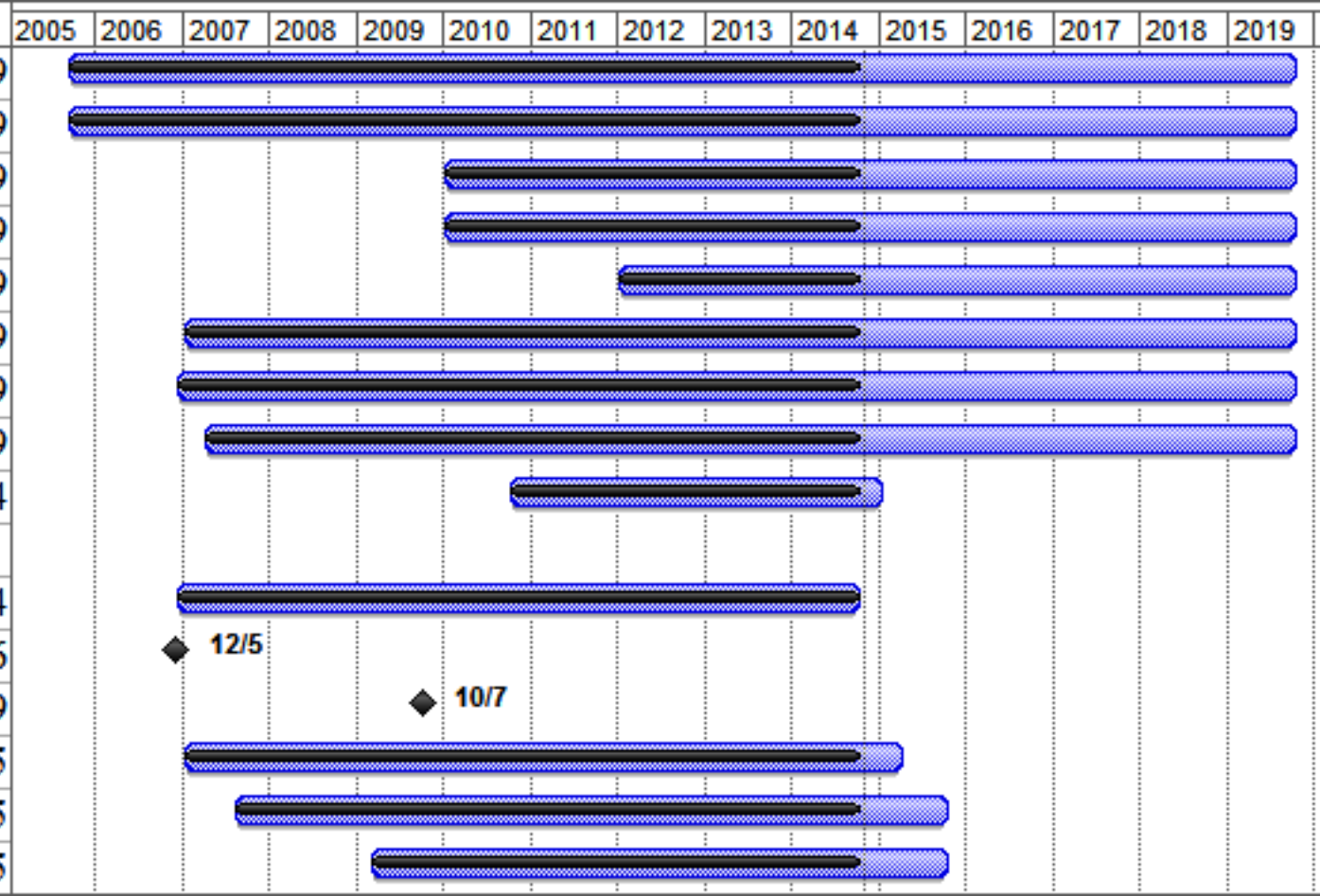
Appendix B
Gantt Charts

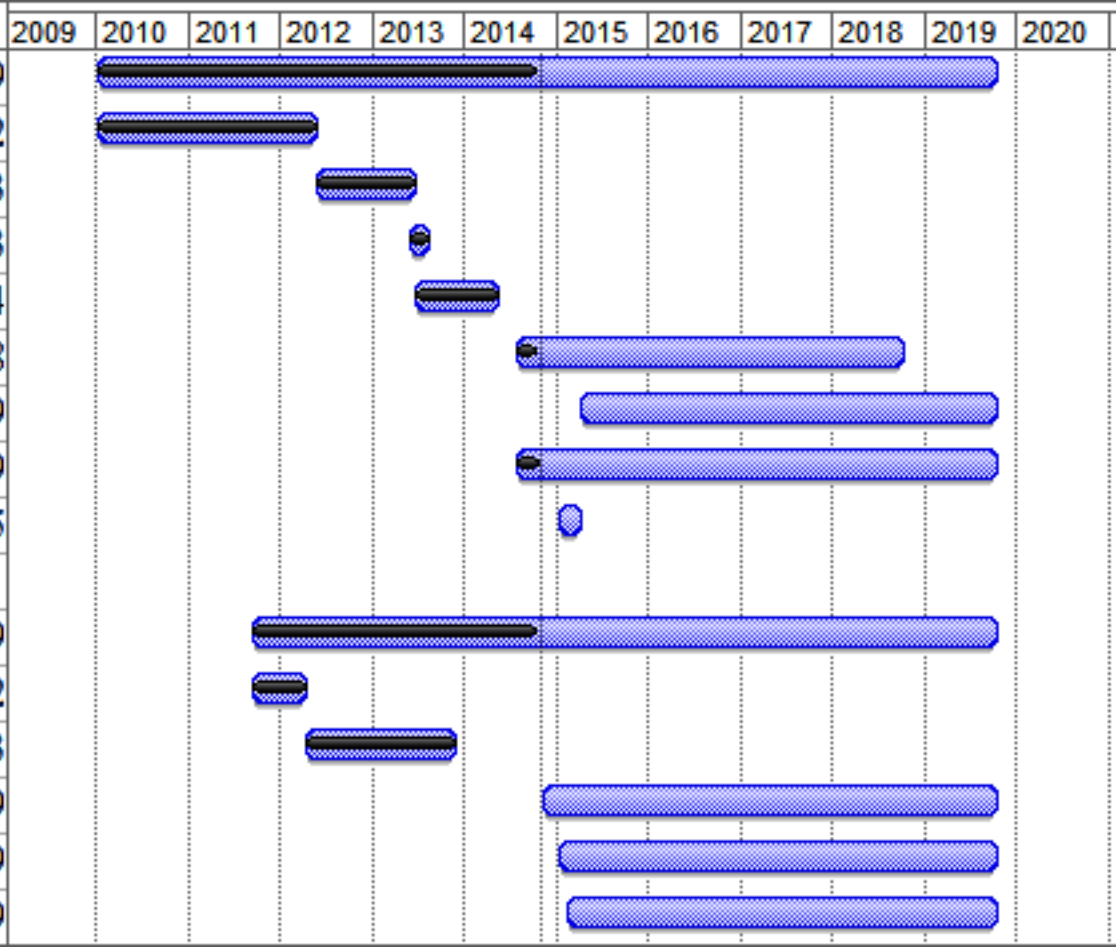
ID	Task Name	Start	Finish
1	Task #1: BATTLE trial - CLOSED	Thu 9/1/05	Sun 3/29/15
2	Presentations and manuscripts	Wed 9/1/10	Sun 3/29/15
3			
4	Task #2: CADRe 5-Yr Follow-up - CLOSED	Wed 3/1/06	Mon 9/29/14
5	IRB protocol approval	Tue 5/23/06	Tue 5/23/06
6	Participant enrollment/Data collection	Fri 2/2/07	Wed 6/30/10
7	Data reconciliation	Fri 10/1/10	Fri 9/30/11
8	Conduct analysis	Wed 12/1/10	Thu 1/31/13
9	Publication plan	Wed 12/1/10	Fri 2/15/13
10	Presentations and manuscripts	Tue 2/1/11	Sun 3/29/15



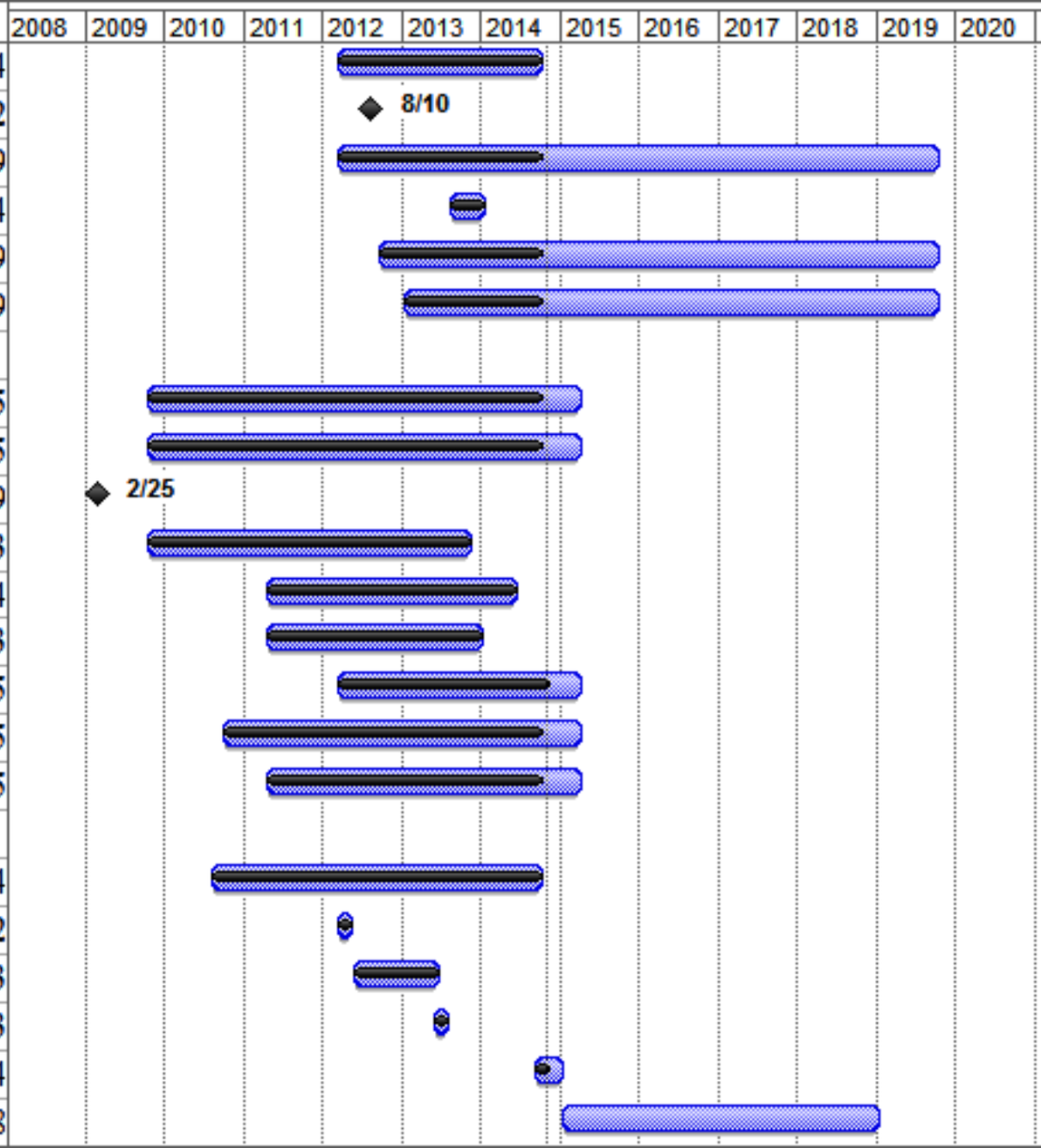
ID	Task Name	Start	Finish
1	Task #3: Continue CPP	Thu 9/1/05	Sun 9/29/19
2	Enrollment/Data collection	Thu 9/1/05	Sun 9/29/19
3	Advance data modeling	Fri 1/1/10	Sun 9/29/19
4	Outreach	Fri 1/1/10	Sun 9/29/19
5	Ultra personal empowering	Mon 1/2/12	Sun 9/29/19
6	Outcomes analysis	Mon 1/1/07	Sun 9/29/19
7	Target subgroup popns	Fri 12/1/06	Sun 9/29/19
8	Presentations/manuscripts	Mon 4/2/07	Sun 9/29/19
9	Upgrade database	Fri 10/1/10	Wed 12/31/14
10			
11	#3.1: Validate CV risk	Tue 12/5/06	Mon 9/29/14
12	IRB protocol approval	Tue 12/5/06	Tue 12/5/06
13	Continuing review approved	Wed 10/7/09	Wed 10/7/09
14	Data collection	Mon 1/1/07	Sun 3/29/15
15	Conduct analysis	Wed 8/1/07	Tue 9/29/15
16	Presentations/manuscripts	Mon 3/2/09	Tue 9/29/15



ID	Task Name	Start	Finish
1	Subtask #3.2: Initiate ZENITH trial	Fri 1/1/10	Sun 9/29/19
2	Protocol development	Fri 1/1/10	Wed 5/9/12
3	Protocol approval WRNMMC/MRMC	Thu 5/10/12	Mon 6/10/13
4	Protocol approval at WMC/MRMC	Fri 5/17/13	Wed 7/31/13
6	Study execution planning	Fri 6/14/13	Wed 4/30/14
7	Recruitment/enrollment/data collection	Tue 7/15/14	Fri 9/28/18
8	Conduct analysis	Wed 4/1/15	Sun 9/29/19
9	Biomolecular studies	Tue 7/15/14	Sun 9/29/19
10	Publication plan	Fri 1/2/15	Sun 3/29/15
11			
12	Subtask #3.3: CPP Prospective Registry	Thu 9/1/11	Sun 9/29/19
13	Protocol development/submission	Thu 9/1/11	Fri 3/30/12
14	Protocol approvals (WRNMMC/MRMC)	Mon 4/2/12	Wed 11/13/13
15	Recruitment/enrollment/data collection	Mon 11/3/14	Sun 9/29/19
16	Data reconciliation/analysis	Fri 1/2/15	Sun 9/29/19
17	Manuscript preparation	Mon 2/2/15	Sun 9/29/19



ID	Task Name	Start	Finish
1	Subtask #3.4: CV Risk in Tramatic Amputations	Thu 3/1/12	Mon 9/29/14
2	Protocol approval	Fri 8/10/12	Fri 8/10/12
3	Recruitment/enrollment/data collection	Thu 3/1/12	Sun 9/29/19
4	Protocol modification for genomics/analytes	Thu 8/1/13	Thu 1/2/14
5	Data analysis	Mon 9/3/12	Sun 9/29/19
6	Presentations and manuscripts	Tue 1/1/13	Sun 9/29/19
7			
8	Task #4: Global Profiling/CRC Completion	Thu 10/1/09	Sun 3/29/15
9	Followup data analysis/publication	Thu 10/1/09	Sun 3/29/15
10	Enroll program participants	Wed 2/25/09	Wed 2/25/09
11	Manuscript on gene expression	Thu 10/1/09	Thu 10/31/13
12	TaqMan SNP analysis	Thu 4/14/11	Fri 5/30/14
13	Metabolite profiling analysis	Thu 4/14/11	Tue 12/31/13
14	Assimilation of PET/CT data	Thu 3/1/12	Sun 3/29/15
15	Conduct molecular analysis	Wed 9/15/10	Sun 3/29/15
16	Presentations & publications	Thu 4/14/11	Sun 3/29/15
17			
18	Task #6: Natural History of Pre-Diabetes	Mon 8/2/10	Mon 9/29/14
19	Protocol development/submission	Thu 3/1/12	Mon 5/7/12
20	Protocol approval at WRNMMC/MRMC	Thu 5/10/12	Tue 6/4/13
21	Protocol approval at WMC/MRMC	Fri 5/17/13	Wed 7/24/13
22	Study execution and planning	Mon 9/1/14	Wed 12/31/14
23	Recruitment/enrollment/data collection	Thu 1/1/15	Mon 12/31/18



ID	Task Name	Start	Finish
1	Task #7: Morbid Obesity	Fri 1/13/06	Sun 3/29/15
2	Protocol approved at WMC	Fri 1/13/06	Fri 1/13/06
3	Protocol approved at TATRC	Fri 6/15/12	Fri 6/15/12
4	Enroll patients	Mon 7/24/06	Fri 8/30/13
5	Obtain blood and tissue samples	Mon 7/24/06	Mon 3/31/14
6	Conduct molecular analysis	Mon 10/1/12	Sun 3/29/15
7	Presentations and manuscripts	Tue 1/1/13	Sun 3/29/15
8			
9	Task #8: Global Long-term	Fri 8/17/12	Sun 3/29/15
10	Protocol approved at WMC	Fri 8/17/12	Fri 8/17/12
11	Protocol approved at TATRC	Thu 5/2/13	Thu 5/2/13
12	Enroll patients	Sat 6/1/13	Tue 4/1/14
13	Obtain blood samples and data	Sat 6/1/13	Tue 4/1/14
14	Conduct molecular analysis	Tue 10/1/13	Sun 3/29/15
15	Presentations and manuscripts	Wed 1/1/14	Sun 3/29/15